*ERRATA*

Volume 33, No. 1, pp. 92 and 93, article on Necrotizing Arteriolitis, should read, "buffered solutions had a pH of 7.4."

Article on Gonadotropic Action of Sera, by E. L. Gustus, R. K. Meyer, and J. H. Dingle, indexed page 255, should read page 257.

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8359 C*

### Immunological Potency of Globulin Fraction as Prepared by Methyl Alcohol Precipitation.

FU-TANG CHU AND CHI-YUAN CHOU.

*From the Division of Pediatrics, Department of Medicine, and the Department of Biochemistry, Peiping Union Medical College, Peiping, China.*

It has been demonstrated that by means of methyl alcohol precipitation fractionation of proteins is possible with sera of dog, ox, sheep and horse and that the protein fractions so obtained can be conveniently converted into dry powder soluble in normal saline.[1] Whether the dry product of such a procedure retains the immune bodies contained therein has not been studied. In this communication is reported the result of experiments on the immunological potency of globulin fraction as prepared by methyl alcohol precipitation. Since human placentas provide a convenient source of pro-

* P represents a preliminary, C a complete manuscript.

1 Liu, S. C., and Wu, H., *Chinese J. Physiol.*, 1934, **8**, 97.

teins with immune substances,[2] they were utilized to supply the globulin fraction in this study.

From 3 to 5 normal placentas were collected in sterile containers into which a measured amount of 1% solution of sodium chloride was previously placed. The placentas were incised and more sodium chloride solution was added, the total volume of saline being 100 cc. for each placenta. After standing in the refrigerator for 48 hours the mixture was decanted and centrifuged to remove the sediment. Several lots were prepared in this way, and the products were pooled and stored at about —13°C. until the time of precipitation.

The globulin fraction was precipitated by methyl alcohol according to the method of Liu and Wu.[1] The precipitation curve in the case of placental saline extract was previously determined, and was found to assume the same general shape as those of the sera of the horse, dog, sheep and ox.

Thirty-five percent methyl alcohol concentration was taken as the separation point for the globulins in the present preparation. Cold methyl alcohol at about —10°C. was added slowly to the saline extract previously cooled to —1°C. The mixture was allowed to stand at —1°C. for 2 hours. The precipitate was separated in a cold centrifuge and dissolved in normal saline. The saline solution thus obtained was poured into 6 times its volume of ether-ethyl-alcohol (3:7) mixture previously cooled to —25°C. and the mixture was allowed to stand at this temperature for 2 hours. The precipitate was filtered, washed 3 times with ether-alcohol mixture and 3 times with ether, sucked dry and then transferred into an extraction thimble. The whole process was carried out at —25°C. in a cold chamber. The globulin was finally extracted in a Soxhlet apparatus with anhydrous ether over sodium for 24 hours.

The dry powder was kept in a desiccator in an ordinary ice chest until it was dissolved in 1% solution of sodium chloride (5 gm. per 100 cc.) shortly before clinical use. About one-fifth of the powder remained undissolved and it was removed by the centrifugation. A 1% solution of merthiolate was added to the final product so as to make the concentration of the preservative 1:5,000.

Intramuscular and intravenous injections of the extract as prepared by the above described method were first carried out in rabbits. No toxic effect was observed. Then a comparison of immunological potency was made between this extract and that which was prepared

[2] McKhann, C. F., and Chu, F. T., *J. Infect. Dis.*, 1933, **52**, 268.

by ammonium sulphate precipitation,[2] both being produced from the same lot of placental saline extract. The following 2 tests were designed for such a comparison.

1. *Neutralization of Dick toxin.* The potency of the 2 preparations derived from the same lots was first compared with regard to the power of neutralizing Dick toxin. Since the antibody which blanches scarlet fever rashes was previously found to be essentially in the pseudoglobulin fraction of the extract,[2, 3] the concentration of the latter was estimated according to the method of Howe,[4] with the assumption that the proteins precipitated at 14 and 22% of sodium sulphate from the extract are the same substances as those precipitated from the whole serum. The pseudoglobulin concentrations were made equal in any 2 preparations which were to be compared. Intradermal tests were then done on the flexor surface of the forearms in Dick positive persons with the mixture of 0.1 cc. Dick toxin and 0.1 cc. of various dilutions of the extracts, the mixtures being first incubated for 30 minutes at 37°C. In order to make the skin tests comparable, one and the same Dick positive person served as the test subject for the 2 different preparations of the same lot. The result of the tests is shown in Table I. It is clear that the titre for neutralization is about the same for the methyl alcohol and ammonium sulphate preparations of the same lots.

TABLE I.
Neutralization of Dick Toxin.

| Dick toxin | Dilution of extract | Lot No. 1 | | Lot No. 2 | | Lot No. 3 | |
|---|---|---|---|---|---|---|---|
| | | $A_1$ | $B_1$ | $A_2$ | $B_2$ | $A_3$ | $B_3$ |
| 0.1 cc. | 1-20, 0.1 cc. | 0 | 0 | 0 | 0 | 0 | 0 |
| " | 1-40, " | 0 | 0 | 0 | 0 | 0 | 0 |
| " | 1-80, " | 0 | 0 | 0 | 0 | 0 | 0 |
| " | 1-160, " | 0 | 0 | 0 | + | 0 | 0 |
| " | 1-320, " | + | + | + | + | 0 | 0 |
| " | 1-640, " | + | + | + | + | + | + |
| " | Saline, " | + | + | + | + | + | + |

$A_1, A_2, A_3$ = Methyl alcohol preparations, of globulin in saline solution, with pseudoglobulin 0.92, 2.71 and 2.70 gm. per 100 cc. respectively.
$B_1, B_2, B_3$ = Ammonium sulphate preparations, diluted to the same pseudoglobulin concentrations as $A_1$, $A_2$ and $A_3$ respectively.
0 = Neutralization.
+ = Redness of more than 1 cm. in any dimension.

2. *Prophylaxis against measles.* In view of the previous experience that measles antibodies seemed to be widely distributed in all the globulin fractions,[3] the concentration of globulin in the saline solution was determined[4] here for the purpose of quantitative com-

[3] McKhann, C. F., Green, A. A., and Coady, H., *J. Pediat.*, 1935, **6**, 603.
[4] Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 109.

parison. The solution of lot 4 as prepared by the methyl alcohol method was given intramuscularly in the dose of 0.28 to 0.36 gm. in 5 children, and the sulphate preparation of the same lot, in the dose of 0.36 gm. in 4. All children were known to have been intimately exposed to measles in their brothers or sisters and none had had measles before. The age of the children and the number of days of exposure before the prophylactic injection was given are comparable in the 2 groups. Either modified measles or complete protection was the result in all the cases.

*Conclusions.* A dry form of placental globulin extract could be prepared by means of methyl alcohol precipitation. When dissolved, this preparation was found to be as potent as the preparation from ammonium sulphate precipitation with regard to neutralization of Dick toxin and prophylaxis against measles.

## 8360 C

## Vaginal Cornification Induced by Swabbing and Its Bearing on the Rat Unit of Estrogenic Substance.

C. K. HU AND C. N. FRAZIER.

*From the Division of Dermatology and Syphilology, Peiping Union Medical College, Peiping, China.*

In the course of a series of assays on one lot of human pregnancy urine extract, employing the method of Coward and Burn,[1] we have encountered findings which were difficult to explain. The recent report of Wade and Doisy[2] directed our attention to the effect of swabbing on the presence of cornified cells in the vaginal smear together with its bearing on the definition of the biological unit of estrogenic substance.

Sexually mature, ovariectomized, albino rats were employed in the experiments. The animals were kept in scrupulously clean cages, each containing 2 or 3 animals. They were fed with a diet in which the supply of vitamin A was adequate. Estrogenic substance was prepared by extracting human pregnancy urine with butyl alcohol by the method described in a previous report.[3] Injections of this extract in olive oil were given by the subcutaneous route.

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[1] Coward, K. H., and Burn, J. H., *J. Physiol.*, 1927, **63**, 270.

[2] Wade, N. J., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 707.

[3] Frazier, C. N., and Mu, J. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 997.

When cornified cells were present in small numbers widely distributed in the smear, or when a few clumps of them were found, the result was marked as $C_1$ (+). A $C_1$ reaction is to be interpreted as borderline positive. Correspondingly greater numbers of cornified cells were graded as $C_2$, $C_3$, or $C_4$ (++, +++, or ++++). The number of leucocytes and nucleated epithelial cells was graded in a similar way. As a rule, the appearance of cornified cells was preceded by a decrease of leucocytes, and followed by a sudden increase of leucocytes, usually with the admixture of nucleated epithelial cells. Full cornification with entire absence of the other cellular elements was of infrequent occurrence. This phase of reaction is at times very transient even when the estrogenic substance is given in oil. Some workers[4] consider the mere disappearance of leucocytes from the smear to indicate a positive reaction, while others[5] require cornification like that of the full estrous smear of the normal animal in which there is a complete absence of leucocytes.

The effect of swabbing alone, carried out 3 or 4 times a day, is manifest in the following observations. Three swabbing experiments were performed on 2 sets of rats. The first set of 24 animals had been previously employed in a series of assays. The second set of 25 animals was used before any estrogenic substance was injected into the animals. The latter group was tested twice with the usual 2 weeks intervening. The results of the 3 experiments were similar enough for combined consideration. Sixty to 88% (average: 78.5%) of the animals gave at least a $C_1$ reaction. Thirty-eight, or 51.3%, of the animals showed a reaction greater than $C_1$.

In a second series of experiments 20 spayed female rats were tested with decreasing doses of the same lot of urine extract. The peak of reaction (the time of appearance of the maximum number of cornified cells) following the injection of 0.5 cc., 0.5 cc., and 0.25 cc. respectively of a 0.5% solution of the extract in olive oil occurred within 40 hours of the injection. Injection of 0.12 cc. or 0.05 cc. of the extract in olive oil, or injection of 0.5 cc. of pure olive oil had their peaks more than 50 or 60 hours after the injection. Actual figures showed that the peak reaction for animals swabbed 3 or 4 times a day without injection of any kind occurred at an average of 46.4 hours after the first swabbing. However, following injection of either estrogenic substance or plain olive oil, a period of about 17 hours elapsed before the first swabbing was made. In order to make

[4] Laqueur, E., and de Jongh, S. E., *J. Pharm. and Exp. Therap.*, 1929, **36**, 1.
[5] Marrian, G. F., and Parkes, A. S., *J. Physiol.*, 1929, **67**, 389.

the different sets of figures comparable, this figure of 17 hours should be added to that of the swabbed untreated animals. The corrected peak reaction would thus be at 63.4 hours. Delayed appearance of cornified cells in the vaginal smears of animals injected with estrogenic substance may therefore indicate a false positive reaction, since swabbing alone causes cornification.

The mechanism of cornification of the vaginal epithelium as induced by swabbing is not clear. Although swabbing apparently stimulates the process of cornification it does not prolong it indefinitely. The cornified cells usually disappear from the vaginal smear within 3 or 4 days after the first swabbing. In general, when epithelial proliferation and cornification follow local irritation, the process is progressive within limits and tends to be chronic.

*Summary.* Swabbing alone produces a significant cornification of the vaginal epithelium in a high percentage of ovariectomized rats. The changes thus induced come later than those following injections of estrogenic substance, and are transient, even though swabbing is continued. The reaction to swabbing materially affects the results of biological assay.

8361 P

## Photodynamic Action of Methylene Blue on Bacteria.

T. T'UNG. (Introduced by Samuel H. Zia.)

*From the Departments of Bacteriology and Immunology and Medicine, Peiping Union Medical College, Peiping.*

The effect of the prolonged use of photodynamically active dyes on a number of bacteria was summarized by Reitz.[1] Workers mixed the dyes and the cultures and let them stand for days exposed or unexposed to light. It was found that some dyes, including methylene blue, were more active in the presence of light than other dyes, such as fluorescein. It was also found that *B. diphtheriae* was more susceptible to such action than *B. typhosus*. Since then, however, very few reports have appeared. In view of the recent interest in the study of photodynamic action on viruses, bacteriophage,[2] and toxins,[3] it seems worthwhile to restudy this problem systematically.

[1] Reitz, Adolf, *Zentralb. f. Bakt.*, 1908, **45**, 270, 374, 451.

[2] Perdrau, J. R., and Todd, C., *Proc. Roy. Soc. London*, 1933, **112**, 277, 288.

[3] Shortt, H. E., and Mallick, S. M. K., *Ind. J. Med. Research*, 1935, **22**, 529. Lippert, K. M., *J. Immunol.*, 1935, **28**, 193.

TABLE I.

| Species of Bacteria | Final Concentration of Methylene Blue | Growth in Exposed Mixture | Growth in Unexposed Mixture |
|---|---|---|---|
| *B. typhosus* | Saturated | ++++ | ++++ |
| | 1-1 | ++++ | ++++ |
| | 1-10 | ++++ | ++++ |
| | 1-100 | ++++ | ++++ |
| | 1-1000 | ++++ | ++++ |
| | 1-10000 | ++++ | ++++ |
| *B. coli* | Saturated | ++++ | ++++ |
| | 1-1 | ++++ | ++++ |
| | 1-10 | ++++ | ++++ |
| *B. dysenteriæ* Shiga | Saturated | ++++ | ++++ |
| | 1-1 | ++++ | ++++ |
| | 1-10 | ++++ | ++++ |
| *B. abortus* | Saturated | + | + |
| | 1-1 | +++ | +++ |
| | 1-10 | +++ | ++++ |
| | 1-100 | ++++ | ++++ |
| *M. catarrhalis* | Saturated | 0 | +++ |
| | 1-1 | 0 | ++++ |
| | 1-10 | ++ | ++++ |
| | 1-100 | ++ | ++++ |
| | 1-1000 | ++++ | ++++ |
| *B. alkaligenes* | Saturated | 0 | ++++ |
| | 1-1 | 0 | ++++ |
| | 1-10 | ++ | — |
| | 1-100 | +++ | — |
| | 1-1000 | ++++ | — |
| *V. choleræ* | Saturated | 0 | 0 |
| | 1-1 | 0 | 0 |
| | 1-10 | 0 | 0 |
| | 1-100 | 0 | 0 |
| | 1-1000 | 0 | +++ |
| | 1-10000 | ++ | — |
| *Staphylococcus albus* | Saturated | +++ | +++ |
| | 1-1 | ++++ | ++++ |
| | 1-10 | ++++ | ++++ |
| | 1-100 | ++++ | ++++ |
| *Streptococcus hemolyticus* | Saturated | — | +++ |
| | 1-1 | — | ++++ |
| | 1-10 | 0 | ++++ |
| | 1-100 | ++ | ++++ |
| | 1-1000 | ++++ | — |
| Pneumococcus type I | Saturated | — | +++ |
| | 1-1 | — | +++ |
| | 1-10 | 0 | ++++ |
| | 1-100 | + | ++++ |
| | 1-1000 | +++ | — |
| | 1-10000 | ++++ | — |
| *B. diphtheriæ* | Saturated | — | ++ |
| | 1-1 | — | +++ |
| | 1-10 | 0 | +++ |
| | 1-100 | 0 | +++ |
| | 1-1000 | +++ | ++++ |

We have studied the effect of short exposures to light of different dilutions of methylene blue on a number of Gram-positive and Gram-negative microorganisms. A preliminary report is presented at this time.

Saturated aqueous solution of methylene blue was diluted to different concentrations with normal saline. Twenty-four hour broth cultures of different organisms were mixed with these solutions in Petri dishes. The bacteria-methylene-blue mixtures were exposed to an ordinary electric light of 100 candle power at a distance of 10 cm. for a period of half an hour. These Petri dishes were placed on a cooling machine which prevents the plate from getting too warm. Soon after the exposure, the organisms were plated out and incubated. The other portions of the same mixtures were not exposed and were similarly plated to serve as controls.

The experimental results are presented in Table I. The photodynamic action of methylene blue on bacteria varies a great deal. In general, the Gram-negative organisms seem to be more resistant than the Gram-positive ones. This parallelism of the reaction with the Gram stain is in conformity with that of bactericidal action of gentian violet in the absence of light[4] and the photodynamic action of a number of dyestuffs on bacteria in the presence of ultra-violet rays.[5]

It has also been found that the antigenicity of Pneumococcus Type I so treated was not lost, and that even after repeated exposures, a culture of *B. diphtheriae* did not lose its virulence. These subjects are now under study.

8362 P

## Effect of Frequency of Stimulation on Tension Response of Muscle.

T. P. FENG.

*From the Department of Physiology, Peiping Union Medical College, Peiping.*

The response of nerve, as measured by its heat production or summated action potential changes, has been shown by Bugnard and

[4] Churchman, J. W., *J. Exp. Med.*, 1929, **16**, 221.

[5] Passow, A., and Rimpan, W., *Müch. Med. Wochr.*, 1924, **91**, 733.

Hill[1] to decline progressively with increasing frequency of stimulation beyond that which calls forth the maximum response. A similar response-frequency relation in the case of muscle is to be expected, but an attempt to obtain it experimentally with toad's or frog's sartorius stimulated by repetitive condenser charges and discharges from a revolving commutator through a pair of silver wire electrodes lying on the tibial half of the muscle, revealed a very different relation. The curve relating the size of isometric contraction in tetani of any duration from 0.44 to 10 sec. and the frequency of stimulation, was found to be a wavy one with recurrent minima and maxima. In an experiment at 26.3°C. with 0.44 sec. tetani the contraction was at a minimum at the approximate frequencies 380, 750, and 1500 per sec. With longer tetani and lower temperatures, the positions of the minima were shifted to lower frequencies.

Further experiments then showed that this strange result was associated with the fact that the stimulating electrodes were laid on the tibial half of the muscle, and that the muscle was partly stimulated through its intramuscular nerve twigs. For this unexpected result was no longer obtained with curarized muscle or when the muscle was stimulated by electrodes on the nerve-free pelvic end. Conversely it was obtained in a much more striking form when the sartorius was excited indirectly through its nerve. The response-frequency curve of muscle when nerve participation is excluded, is a smooth one, and for short tetani generally very flat after the maximum, unless complicated by fatigue.

An explanation may be offered on the following basis: First, assume that the neuromuscular junction has the property of a narcotized region having a somewhat longer refractory period than the nerve and in which conditions for the development of Wedensky inhibition exist, in particular a condition in which ineffective stimuli falling in the relative refractory period leave an inhibitory after-effect, while those extinguished in the absolute refractory period do not.[2] Second, assume progressive failure of excitation to occur in the intramuscular nerve twigs with increasing frequency of stimulation beyond the maximum, as shown by Bugnard and Hill in isolated nerve trunks. Referring to the experiment at 26.3°C. quoted above, it may be supposed that when the somewhat subnormal impulses impinge on the junctional tissue at the frequency of 300-400/sec., they will be largely made ineffective in its relative refractory phase, each leaving an inhibitory after-effect, producing

[1] Bugnard, L., and Hill, A. V., *J. Physiol.*, 1935, **83**, 383.

[2] Kato, G., Nakayama, M., and Ota, T., *Am. J. Physiol.*, 1929, **90**, 406.

Wedensky inhibition, and thus giving rise to the first minimum. As the nerve impulses arrive with frequencies higher than 400/per second, about half of them would be extinguished in the absolute refractory period, leaving no inhibitory after-effect, and the other half coming later in the relative refractory phase would have greater chance of becoming effective. Hence the response-frequency curve rises again. But now as the stimulating frequency is increased beyond 500-600 per second, which is probably the maximum rhythm the nerve can follow, the propagated number of impulses per sec. actually become less and less with further increase of frequency of stimulation. When this number has dropped to 400-300 per second, Wedensky inhibition would again occur, hence a second minimum. With further drop in the impulse frequency the response frequency curve would naturally rise again, and would then fall a third time when impulse frequency becomes smaller than that necessary for calling forth the maximum contraction. The subsequent rise in the curve cannot be understood according to this scheme, but in this region of frequency of stimulation, summation of subliminal stimuli would probably occur and more muscle fibres become directly stimulated.

## 8363 P

### Use of Saturated Sodium Chloride Solution for Preservation of Viruses.

F. C. LIN, T. J. KUROTCHKIN AND C. V. BENARADSKY.

*From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, and the Kalgan Veterinary Laboratory, Kalgan, China.*

There are very few convenient methods for the preservation of viruses. The use of disinfectants is not permissible because of their deleterious effect upon viruses, while the commonly employed glycerol exerts only a feeble antiseptic action and, therefore, cannot be relied upon when freedom from bacterial contamination is important. The preservation of viruses through the process of drying *in vacuo* at low temperatures is an excellent method but it has the drawback of being somewhat cumbersome.

It has been shown by Ginsburg and Kalinin,[1] Sonnenschein,[2] and

[1] Ginsburg, S., and Kalinin, W., *Z. f. Immunität.*, 1929, **63**, 107.

[2] Sonnenschein, C., *Z. f. Immunität.*, 1930, **66**, 330.

others, that 10% solution of sodium chloride is effective in preserving complement for about 4 weeks. This action of sodium chloride upon complement, a substance of known instability, suggested to us that this salt may be a good agent for the preservation of viruses.

We found that saturation of guinea pig complement with sodium chloride preserves it for at least one month, and that various bacteria of the Salmonella group, pneumococcus, meningococcus, gonococcus, *Streptococcus hemolyticus* and *viridans, Staphylococcus aureus, B. influenzae* and *B. diphtheriae* are killed in from a few days' to one month's time when kept in saturated solution of sodium chloride.

For the present work we selected rinderpest and vaccine viruses and *B. dysenteriae* Shiga bacteriophage. The preservation of the first named virus is frequently a matter of great difficulty, especially in warm seasons, and, therefore, a method permitting its preservation even for a few weeks would obviously be of practical importance.

*Rinderpest virus.* A young Mongolian calf was artificially infected with rinderpest and killed at the height of febrile reaction. Pieces of spleen, measuring 5x3 cm., were placed in a glass stoppered bottle containing 100 cc. of saturated sodium chloride solution and kept in the ice chest. After 2 weeks a thick, coarse suspension of splenic tissue was prepared by grinding with saline and one cc. was injected subcutaneously into a normal young calf. Four weeks later 2 normal calves were similarly inoculated. All 3 animals developed typical rinderpest infection, with the usual incubation period of 3-4 days. Their blood was infectious for 3 normal calves. On necropsy these animals presented extensive ulceration of the abomasum. Clinically and pathologically the infection was associated with changes similar to those found in animals infected with fresh virus. This suggests that no (qualitative?) attenuation of the virus took place after 4 weeks of contact with saturated solution of sodium chloride.

*Vaccine virus.* Calf lymph was ground in a mortar with a small amount of 0.9% saline to form a thick paste. One-half was preserved by mixing with 4 times its weight of a solution containing 60 parts of glycerol and 40 parts of 1.8% phenol. The other was suspended in a similar quantity of distilled water saturated with sodium chloride. Each lot of vaccine was then subdivided, one portion being kept in the ice chest and the other at room temperature. Determination of their potency was performed at different time in-

tervals for 5 weeks. A rabbit was given intradermally 0.2 cc. of identical dilutions of 2 vaccines stored under similar conditions. During a storage of 5 weeks in ice chest, the titer of the vaccines gradually dropped from 1:10,240 to 1:1,280. No difference in the potency of glycerol and saturated salt solution vaccines was noted. Titration of vaccines kept at room temperature showed that during a period of 5 weeks the titre of glycerol vaccine decreased from 1:10,240 to 1:160 and that of saturated salt solution to 1:320. This slight difference was considered insignificant, although somewhat more intense reactions resulted from the vaccine preserved in saturated sodium chloride solution.

*Dysentery Shiga bacteriophage,* titre $1x10^{-10}$, was treated with an excess of sodium chloride and with an untreated control, distributed into tubes, sealed with paraffine and placed at 37°C. Titration of both lots of phage was performed from time to time for 3 months, by photometric measurements of turbidity[3] of young cultures of *B. dysenteriae* Shiga in broth to which phage was added. At the end of 3 months the potency of control phage had fallen from $1x10^{-10}$ to $1x10^{-5}$, while that of the salt-preserved phage dropped only to $1x10^{-8}$. This difference indicates that sodium chloride has a definite value in preserving the potency of phage. Its favorable effect has been further demonstrated by determination of the incubation time (time interval between the addition of phage and onset of bacterial lysis), using dilutions varying from $1x10^{-4}$ to $1x10^{-10}$. At the end of 3 months the incubation time of any dilution used in case of phage preserved with sodium chloride was 16 minutes shorter than that of the control lot. This indicates that the potency of salt-preserved phage was 400 times higher than that of the control phage.

## 8364 C

## Use of Chinese Hamster for Testing the Virulence of C. Diphtheriae.

K. J. LU AND SAMUEL H. ZIA.

*From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.*

The use of animals for the determination of virulence of *C. diphtheriae* is often necessary in the study of cultures obtained from patients or suspects. Frequently it is also desirable to use animals in

[3] Lin, F. C., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 488.

certain epidemiological studies, *e. g.,* in determining the incidence of carriers. To employ guinea pigs in large numbers is expensive. In order to find a cheaper laboratory animal, the Chinese hamster *Cricetulus griseus*, has been selected. Hamsters (field mice) are easily procurable in this part of the world and their susceptibility to diphtheria toxin has been reported from this laboratory.[1] The purpose of the studies here reported was to conduct comparative tests to determine the reactions of hamsters and of guinea pigs to cultures of *C. diphtheriae* recently isolated from patients. It is necessary to mention in this connection that as the hamsters are readily susceptible to pyogenic infections, pure cultures of diphtheria organisms are prerequisite in using these animals. Fortunately the use of simple tellurite blood agar plates[2] readily yields a pure culture of this group of organisms.

*Preparation of bacterial emulsions.* Isolated cultures were separated into 3 groups: (1) Pure cultures of typical *C. diphtheriae,* with bulging polar bodies; (2) cultures with only a few of the organisms carrying typical polar bodies, and (3) cultures of diphtheria-like organisms without polar bodies. The morphology of the organisms was determined in each instance before the cultures were injected into animals. Pure cultures were obtained by plating on 0.04% potassium tellurite blood agar plates and single typical colonies were picked. The culture was then grown on Loeffler's slant for 24 hours and the growth was emulsified in 2 cc. of normal saline. Of this emulsion 0.2 cc. was injected into guinea pigs and 0.1 cc. into hamsters.

*Inoculation of guinea pigs.* The usual intradermal technique was followed. A control animal received 1000 units of antitoxin 24 hours before, while the test animal received 250 units 4 hours after the injection of organisms. Five to 7 tests may be made on one large-sized guinea pig. Readings were made daily for 3 days. With the exceptions to be noted, frank necrosis, at the site of inoculation of the virulent cultures, was produced invariably whereas the control animals remained entirely normal.

*Inoculation of hamsters.* Hamsters weighing from 20 to 30 gm. were used. Control animals received intraperitoneal injections of 50 units of antitoxin 24 hours prior to the subcutaneous injection of cultures. The animals were kept in separate cages and were observed for 5 days. It was found that test animals injected with

[1] Fan, C., and Lim, C. E., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 226.

[2] Horgan, E. S., and Marshal, A., *J. Hyg.*, 1932, **51**, 441.

virulent cultures usually died within 50 hours, most of them before 40 hours. Autopsy of these hamsters revealed only adrenal congestion. Those that survived 5 days were discarded.

*Results.* One hundred and two different cultures recently isolated from patients were thus tested. The results are presented in Table I.

TABLE I.
Comparison of Results in Diphtheria Virulence Tests on Guinea Pigs and Hamsters.

| Morphology | Virulence | Total No. | No. in agreement | No. not in agreement In favor of guinea pig | No. not in agreement In favor of hamster |
|---|---|---|---|---|---|
| Typical | Virulent | 51 | 48 | 2 | 1 |
| | Avirulent | 9 | 9 | | |
| Atypical with few polar bodies | Virulent | 6 | 6 | | |
| | Avirulent | 13 | 12 | | 1 |
| Diphtheria-like organisms | Virulent | 3 | 1 | 1 | 1 |
| | Avirulent | 20 | 19 | | 1 |
| Total | | 102 | 95 | 3 | 4 |

It can be seen from the table that the results obtained were fairly comparable. Two out of 3 discrepancies in which guinea pigs excelled were due to the death of the control and test hamsters. This might have been obviated if more than one set of animals had been used for each specimen. It may be noted that the use of guinea pigs was not entirely without error; in 2 instances, control pigs showed definite necrosis, in one, the test animal failed to react, and in a fourth, the test animal showed a reaction to a non-virulent culture. All these have been checked with the result that the guinea pigs agreed well in all 7 instances with the hamsters.

*Discussion and Summary.* The experiments demonstrate that hamsters react much in the same way as guinea pigs to cultures of *C. diphtheriae* or of diphtheriae-like organisms. There was complete agreement in 95 out of 102 instances. To use hamsters instead of guinea pigs possessed the advantage of giving clear-cut results because these animals invariably died from the virulent organisms and survived the non-virulent ones. Equivocal results from intradermal tests on guinea pigs due to secondary infection from field cultures, or to injections being made too deep into the skin, could thus be avoided.

Other advantages are that 10 hamsters may be purchased for the cost of one guinea pig, and that the control hamsters require much

less antitoxin. Objections may be raised in that pure cultures are necessary, thereby causing possible delay. This is only of theoretical interest, because usually it is only in doubtful and convalescent cases of diphtheria that this test is required, and a delay of one day does not limit its usefulness. Furthermore, one must remember that in making the conventional test on guinea pigs a delay of 24 hours is also necessary after the control guinea pig has received antitoxin.

As a result of these studies it has been shown that Chinese hamsters are suitable animals for the routine determination of the virulence of *C. diphtheriae*. The use of these animals instead of guinea pigs is very much less expensive and in certain respects gives more reliable information.

8365 P

## Photodynamic Action of Methylene Blue on Diphtheria Toxin.

F. C. LIN. (Introduced by Samuel H. Zia.)

*From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping.*

The effect of photodynamic action of certain dyes on various substances, among which may be mentioned different types of animal cells, viruses[1, 2, 3] and soluble toxins of both bacterial[4] and animal origin,[5] is a well-known phenomenon. Workers generally agree as to the extreme susceptibility of these substances to the photodynamic action of dyes, the optimal concentration of the dye employed, and the relatively short time required for exposure, but disagree with regard to the antigenicity of substances so treated. The present study aims to investigate the reaction of diphtheria toxin to the photodynamic action of methylene blue upon which subject little study has been made. Diphtheria toxin is looked upon as a more suitable material than either tetanus toxin or viruses on account of its greater stability and ease with which quantities given can be accurately measured and properly controlled.

The technique employed is essentially similar to those of other workers.[1, 5] Methylene blue is selected since it has been more thor-

[1] Perdrau and Todd, *Proc. Roy. Soc. Bull.*, 1933, **112**, 277, 288.
[2] Perdrau and Todd, *J. Comp. Path. and Therap.*, 1933, **46**, 78.
[3] Shortt and Brooks, *Indian J. Med. Res.*, 1934, **21**, 581.
[4] Lippert, *J. Immunol.*, 1935, **28**, 193.
[5] Shortt and Mallick, *Indian Med. J. Res.*, 1935, **22**, 529.

oughly studied than other dyes. A single batch of toxin containing 400 M.L.D. per cc. for the guinea pig, or 2000 M.L.D per cc. for Chinese hamsters weighing 20-25 gm., was used throughout the experiment, employing as diluent 0.9% salt solution for both dye and toxin. As source of radiation, both direct sunlight during summer and early autumn and electric light of 100 watts, 110 volts at 16.5 inches distance were used. In a preliminary study, the minimal time for exposure was found to lie between 20-30 minutes. In order to obtain full manifestation of combined action of light and dye, it was subsequently fixed at 60 minutes in all instances. Table I shows the maximal M.L.D. for hamsters which may be destroyed by the optimal concentration of methylene blue when exposed to sunlight and to artificial light. The number of animals in each group varied from 3 to 9.

TABLE I.

| Methylene blue<br>Toxin in M.L.D. for hamsters per cc. | 1:100 | 1:1,000 | 1:10,000 | 1:100,000 |
|---|---|---|---|---|
| | Exposure to Sunlight, 60 Min. | | | |
| 10 | S | S | S | S |
| 25 | — | S | S | S |
| 50 | D | S | S | S |
| 100 | D | S or D | S | S |
| 200 | D | D | S | D |
| 400 | D | D | D | D |
| | Exposure to Electric Light, 60 Min. | | | |
| 10 | — | D | S | S |
| 20 | — | D | S | S |
| 30 | — | D | S | S |
| 40 | — | D | S or D | S |
| 50 | — | D | D | D |

D = death within 3 days after inoculation.
S = survived.
— = not done.

Control hamsters receiving unexposed toxin plus dye or exposed toxin without dye invariably succumbed in 3 days or less. From the table it is seen that the combined action of dye and sunlight is greater than that of dye and artificial light. Subsequently it was found, however, that the photodynamic action of dye did not inactivate toxin completely, since it was possible to detect by both intracutaneous test on guinea pigs and subcutaneous inoculation of increased quantities in hamsters, traces of toxin after exposure either to sunlight or artificial light in the presence of methylene blue.

8366 C

# Experimental "Constant Oestrus" and the Notion of Anti-Gonadotropic Hormones.*

G. P. DU SHANE, W. T. LEVINE, C. A. PFEIFFER AND E. WITSCHI.

*From the State University of Iowa.*

In the recent literature frequent references[1, 2, 3] occur to refractoriness of the ovary toward prolonged or repeated stimulation with urinary and pituitary hormones. In many instances the unresponsiveness of an overstimulated ovary is due, partly, to extreme luteinization, follicular exhaustion or other degenerative changes. Moreover, the hypophysis of the injected or implanted animal undergoes transformations, mostly of a depressive nature. These 2 factors account as a rule for non-return to the normal condition immediately after experimental procedures, Hertz and Hisaw.[3]

Collip and his coworkers[4, 5] have shown that the blood of rats which have become unresponsive to urinary hebin is able to inactivate this hormone. Such a serological reaction may be interpreted either as due to the appearance of antibodies to the foreign (human) proteins, or to the increase in "antihormones" normally controlling hormonic levels in the blood. Collip[4] prefers the second alternative and Bachman[6] has attempted to rule out the first one, though obviously without success.

In view of the fact that the refractory condition has been obtained by the introduction of foreign proteins (mouse, rat, rabbit as recipients; man, horse, cattle, sheep as donors) immunity reactions should be expected *a priori*. That animals became "insensitive" even by injection of subthreshold doses of hormones, and remained so for months afterwards (Hertz and Hisaw[3]) further suggests an immunological interpretation. Therefore it becomes necessary to test whether the refractory state may be established also by gonadotropic

* Aided by grants from the National Research Council, Committee for Research in Problems of Sex.

1 Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 487.

2 Hill, R. T., Parkes, A. S., and White, W. E., *J. Physiol.*, 1934, **81**, 335.

3 Hertz, R., and Hisaw, F. L., *Am. J. Physiol.*, 1934, **108**, 1.

4 Collip, J. B., *Mount Sinai Hosp. Reports*, 1934, 1.

5 Selye, H., Bachman, C., Thomson, D. L., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1113. Bachman, C., Collip, J. B., and Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 544.

6 Bachman, C., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 851.

substances derived from identical species. Experiments of long duration with parabiotic rats, offer probably the best attainable conditions to solve this question. From colorimetric data published by Hill[7] we calculated that almost 0.1 cc. of blood per minute or 150 cc. per day are exchanged between the members of a pair. Since unoperated females thus connected run oestrus cycles independently and may become pregnant even after long duration of parabiosis we must conclude that the hormone levels in normal animals are at all times relatively low and also that hormones disappear quite rapidly from the circulation. These two facts are drastically brought out by pairs in which one member is hypophysectomized. If the hypophysis is removed at an early date (50 to 60 days) the deprived member soon lags in body size. After puberty the normal member starts regular oestrus cycles, has normal ovaries, thyroids and adrenals, while the hypophysectomized twin has ovaries less than 20 mg. in weight, undeveloped accessory genital organs, and the adrenals

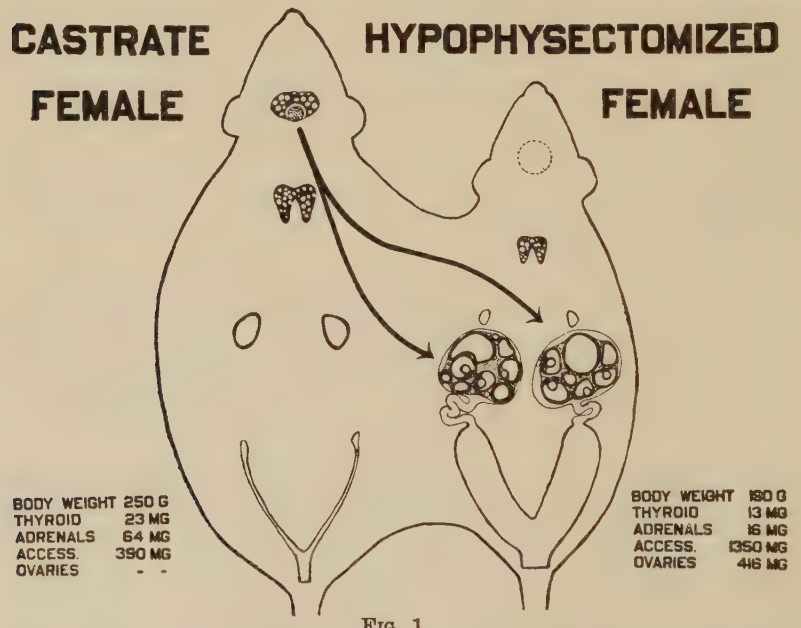


FIG. 1.

Diagrammatic representation of hormonal reactions in a pair of parabiotic female rats. *1st operation at 30 days:* litter mate females united in parabiosis. *2nd operation at 50 days:* hypophysectomy in right member (retardation of growth, reduction of thyroids, adrenals and genital system). *3rd operation after puberty:* castration of left member (reduction of genital ducts and excessive production of follicle-stimulating hormone in the castrate; exaggerated constant oestrus in the hypophysectomized twin.)

[7] Hill, R. T., *J. Exp. Zool.*, 1932, **63**, 203.

as well as the thyroids are of the reduced type characteristic for single hypophysectomized animals. In short, 150 cc. daily of normal rat blood do not carry enough gonadotropic, thyreotropic, adrenotropic, growth or oestral hormones to relieve any of the deficiencies caused by hypophysectomy. This picture changes at once if the normal twin is castrated (Fig. 1). The ovaries of the hypophysectomized animal begin to develop, follicles with antra become rapidly prominent and within 4 to 6 days the animal establishes a condition of constant oestrus. Of the many cases of this type among our parabiosis series we mention pair 192 (male castrate + female hypophysectomized), constant oestrus observed 3½ months; pair 199 (female castrate + female hypophysectomized), constant oestrus observed 5 months; pair 221 (female castrate + female hypophysectomized), constant oestrus observed 4 months. Particularly interesting is pair 208. Originally a combination of a male castrate with a hypophysectomized female the latter had been in constant oestrus for 3 months when she was castrated. The ovaries removed were of the extraordinary size characteristic for these combinations (416 mg., without capsule or oviduct). Uteri, vagina and vestibular glands were correspondingly enlarged. Following castration the female, naturally, fell in anoestrus. After 2½ months the ovaries of a newborn rat were implanted into her kidneys with the result that 20 days later oestrus reappeared, soon became constant and thus persisted for 5 months, until the animal was killed. Two of the present authors have shown[8] that the constant oestrus condition is due entirely to the influx of follicle stimulating hormone from the hypophysis of the castrate twin. This hormone obviously is the only one transmitted, for corpora lutea are never formed, thyroid and adrenals do not recover nor is body growth resumed.

It has been shown that constant oestrus is not only established by hypophysectomized females but also by normal ones if they are parabiotically united with castrated or with otherwise sterilized mates. However, the female in possession of its hypophysis goes into constant oestrus only after a certain period of irregularity, during which the capacity of the normal female hypophysis to release luteinizing hormones gradually becomes suppressed. Table I contains some cases in which constant oestrus has been observed for periods of several months. In these and in over a hundred more pairs of similar constitution, studied during the past years, constant oestrus never came to an end spontaneously. Sooner or later the

[8] Witschi, E., and Levine, W. T., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 101.

mucosa of the uteri and even oviducts and ovaries may become infiltrated with leucocytes. Therefore, leucocytes appear frequently besides cornified cells in the smears in later months of constant oestrus. While the smear resembles then stage IV, sections through the vaginal epithelium still give the picture characteristic of stage III. Shrinkage of the uteri and disappearance of cornified cells from smears follow presently after hypophysectomy in the co-twin, separation of the pair or castration of the female exhibiting the constant oestrus condition. Females that have been in constant oestrus for several months resume cyclical oestrus after separation from their co-twin. At the beginning these cycles are extremely prolonged and irregular. Fertility tests have not been carried out in a systematic way, though our records show that the females of the pairs 145 and 153 (Table I) became pregnant 4 and 3 months after separation from their sterile co-twins.

TABLE I.

Oestral Reactions in Unoperated Females Which Are United in Parabiosis with Castrated or Sterilized Cotwins.

| No. | 1st twin | 2nd twin | irregular period* months | constant oestrus months |
|---|---|---|---|---|
| P111 | ♂ castrate | ♀ unoperated | 3 | 7½ |
| P115 | ♂ " | ♀ " | 1½ | 14½ |
| P205 | ♀ " | ♀ " | 3 | 10½ |
| P145 | ♀ X-rayed | ♀ " | 1 | 5 |
| P151 | ♀ " | ♀ " | 1½ | 4½ |
| P153 | ♀ X-rayed (later castrated) | ♀ " | 1 | 4½ |
| P175 | ♀ X-rayed | ♀ " | 1 | 4 |
| P149 | ♂ " | ♀ " | ½ | 6½† |
| P157 | ♂ " | ♀ " | 1 | 10‡ |
| P161 | ♂ cryptorchid | ♀ " | 2 | 8 |
| P178 | ♂ " | ♀ " | 1 | 4 |

*Concerning the factors responsible for this period see Witschi and Levine.

†With an interruption in the third month, probably due to partial regeneration in the testes of the cotwin.

‡With an interruption in the third month; after a renewed X-ray treatment of the testes constant oestrus was resumed.

The amount of follicle-stimulating hormone received by these parabiotic "constant oestrus females" is not exactly known. Injection experiments indicate that it is far below 150 daily rat units. On the other hand, it is above the single unit. In consideration of the effect on the ovaries of hypophysectomized females one may venture an estimate of about 5 to 20 daily units. At any rate it is clear that the ovaries are constantly being overstimulated. The high level in follicle-stimulating hormone is maintained because the oestrin is not transmitted in physiological quantities to the sterile member. This

is evident from the castrate condition of vagina and uterus in the sterile co-twin (Fig. 1). Even daily injections of as many as 100 rat units of oestrin in one member of a pair do not bring about oestrus in the ovariectomized co-twin. Obviously oestrin disappears so rapidly from circulation that it can not reach a sufficient concentration, in the sterile member, to check the increased hypophyseal activity.

Recently one of us (Pfeiffer[9]) has developed another method of constant follicular stimulation in female rats. Newborn rats are implanted with testes of male litter mates. In cases of successful takes their hypophyses assume the male character of secreting constantly follicle-stimulating hormone and not releasing any luteinizing hormone even in the presence of considerable amounts of oestrin in the circulating blood. Constant oestrus, therefore, is established in these rats as soon as they reach the age of puberty. In contrast to the described situation in the parabiosis experiment, the ovaries in this case are not overstimulated and oestrus is of a low grade, producing cornification in the vagina but only a very moderate enlargement of the uteri. Apparently the activity of the hypophysis is kept on a moderate level by the oestrin from the ovaries. A state of equilibrium between hypophysis and ovaries is thus established, which resembles very much the normal oestrus condition in the rabbit. This constant oestrus condition has been observed for periods of 4 or 5 months in many of these implanted females. Neither luteinization nor ovulation ever occur spontaneously and all indications of cyclical processes are wanting. After a 4 to 5 month period of continuous oestrus 10 of these females were injected, each with 4 mg. of a purified extract of the luteinizing hormone from powdered sheep pituitary (equivalent of 120 mg. dry powder). All of them ovulated within 10 hours, each ovary releasing 4-9 perfectly normal eggs (Witschi and Pfeiffer[10]). One must conclude therefore that the ovaries had constantly maintained about 5 eggs at the proper stage to be released and to proceed presently through the final maturation stages, upon the stimulus provided by a given amount of luteinizing hormone.

In a further series of experiments with these rats, it was found, however, that the ovulation reaction can not be repeated with the sheep luteinizing hormone. Even more than one month after a single injection or after repeated treatments, the rats are unrespon-

[9] Pfeiffer, C. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 603.

[10] Witschi, E., and Pfeiffer, C. A., 1935, *Anat. Rec.*, in press.

sive, *i. e.*, remain in constant oestrus. On the contrary, if the treatment is repeated with extracts from human pregnancy urine (4-10 cc.) ovulation follows the injection within 12 hours (3 cases).

These experiments prove irrefutably that continuous stimulation of the rat ovary with moderate or with large amounts of follicle-stimulating hormone results in a state of constant oestrus which may persist for more than a year, provided that this hormone has been derived from the same species. It is concluded that the loss of sensitivity of ovaries of rats and rabbits after injection of gonadotropic hormones of heterogeneous origin is of the nature of an immunity reaction. The fact that the gonadotropic substances so far isolated are proteins will of necessity set certain limits to the possibilities of therapeutic application of extracts from animal tissues. No evidence of the presence of specific anti-gonadotropic substances in the sense of Collip was found. The fact that the ovaries of hypophysectomized females in parabiosis with castrates enlarge to about 4 times the weight of those of females in possession of the hypophysis indicates that the hypophysis normally not only stimulates but also limits the follicular growth in the ovary. Preliminary experiments suggest that the thyroids and possibly also the adrenals enter as factors in this complex mechanism of control.

Of great interest in this connection is also the recent report by Wade and Doisy[11] on the prolonged administration of oestrogenic hormones (theelin and theelol) to male and female rats. These investigators find that the injury to the ovary produced by short period treatments becomes largely repaired after prolonged treatment. Female rats injected daily with varied (partly with excessive) amounts of female sex hormone resume nearly normal cyclical oestrus and become pregnant like normal controls. Even spayed females do not stay in constant oestrus but run "irregular cycles of 5 to 10 days' duration interspaced with long periods of oestrus and dioestrus up to over 30 days." From their observations the 2 authors draw the following conclusion: "Since the quantity of hormone administered was kept constant it would appear that there is a rhythmic response in the cells sensitive to the hormone." This inference seems unwarranted in view of the fact that not only the vaginal, but obviously also the ovarial cycles are maintained in spite of the injections. On the other hand, our own experiments show that vaginal oestrus becomes continuous if the ovaries are in the constant oestrus condition—no matter whether moderately or

[11] Wade, N. J., and Doisy, E. A., *Endocrinol.*, 1935, **19**, 77.

strongly stimulated. On the basis of the available data it would seem not unlikely that the oestrin-injected animals develop some means to dispose more rapidly of the injected dosages of hormone, probably by excretion, so that they have little chance to evoke physiological reactions in the genital organs.

*Summary.* Parabiotic twins of rats exchange daily about 150 cc. of blood. If one member of a female pair is hypophysectomized it exhibits deficiencies which prove that blood received from the normal mate carries not enough hormones to maintain body weight, thyroids, adrenals and ovaries at normal levels. Castration or sterilization of males and females increases the output of follicle-stimulating hormones to the extent that normal or hypophysectomized twin females go into a condition of constant oestrus. The ovaries are visibly overstimulated and contain large follicles but no corpora lutea. Uterus and vagina exhibit also the characteristics of a maximal or even an exaggerated oestrus condition, indicating the release of large amounts of oestrin by the ovaries. Not enough oestrin accumulates in the castrate member to prevent complete atrophy of the secondary sex characters. This indicates rapid disappearance even of high amounts of oestrin from the circulation. Constant oestrus with a moderate degree of ovarian and vaginal stimulation and almost dioestral size of the uterus is observed in females with experimentally masculinized hypophyses. Both types of constant oestrus can persist indefinitely, at any rate over a full year, without loss of sensitivity by either ovaries or genital accessory organs. It is concluded that no specific anti-hormones are formed, though the hypophysis, probably through the intermediary of the thyroid (and adrenals?) checks the excessive growth of overstimulated ovaries. The loss of reactivity in ovaries after prolonged stimulation with gonadotropic hormones of heterogeneous origin is considered as an immunity reaction to foreign proteins.

8367 P

# A Thermostromuhr Operating on Storage-Battery Current.*

CARL F. SCHMIDT AND ARTHUR M. WALKER.

*From the Laboratory of Pharmacology, University of Pennsylvania.*

This method is an attempt to secure the advantages of the thermostromuhr devised by Rein[1] without the complications attendant upon the quantitative application of high-frequency currents to living animals. The design and construction were arrived at by empirical trials on a schema. The actual testing and calibration were done entirely upon living animals.

The thermostromuhr element is incorporated in a bakelite block; it closely resembles that of Rein in external appearance and dimensions (Fig. 1). The upstream or cold junction (A) and the down-

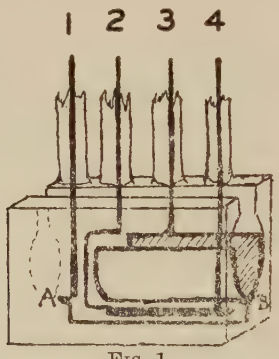


FIG. 1.

stream or hot junction (B) are silver-constantan and lie uncovered at the bottom of a trough 2-2.5 mm. in diameter. The hot junction (B) is at the downstream end of a semicylindrical plate of silver 5 mm. long and 0.04 mm. thick. Under this plate, insulated from it by a thin layer of mica, lies a strip of nichrome about 1 mm. wide, 0.04 mm. thick, and 5 mm. long; copper leads (2 and 4) carry to it, through a rheostat and ammeter, the current from a 6-volt storage battery. The thermocouple wires (1 and 3) are of silver; they are connected to a galvanometer along with a suitable dry-cell-potentiometer system to permit convenient setting of the galvanometer image

* The expenses of this investigation were defrayed in large part from grants by the Commonwealth Fund, and the Faculty Research Fund, University of Pennsylvania.

[1] Rein, H., *Z. f. Biol.*, 1928, **87**, 394.

on the scale. When a blood-vessel is placed in the trough and the silver plate is heated by passing a suitable current (2 to 2.5 amperes) through the nichrome strip, the extent to which the silver plate is cooled by the blood stream varies directly with the volume of blood-flow. With our apparatus a change of 1°C. in the temperature difference between the two junctions causes a galvanometric deflection of approximately 10 cm.; this represents an E.M.F. change of approximately 0.045 millivolt.

Calibrations were made with the element installed on the renal veins of 17 rabbits and on the abdominal aortas of 5 rabbits, by comparing the galvanometric readings with the outflow from the renal vein as measured by the method of Barcroft and Brodie.[2] The animals were eviscerated and the abdominal circulation was confined to the left kidney. Renal blood-flow was altered by intravenous injections of hypertonic glucose, sulphate, and urea, and by bleeding and transfusion. If the blood-vessel completely covered

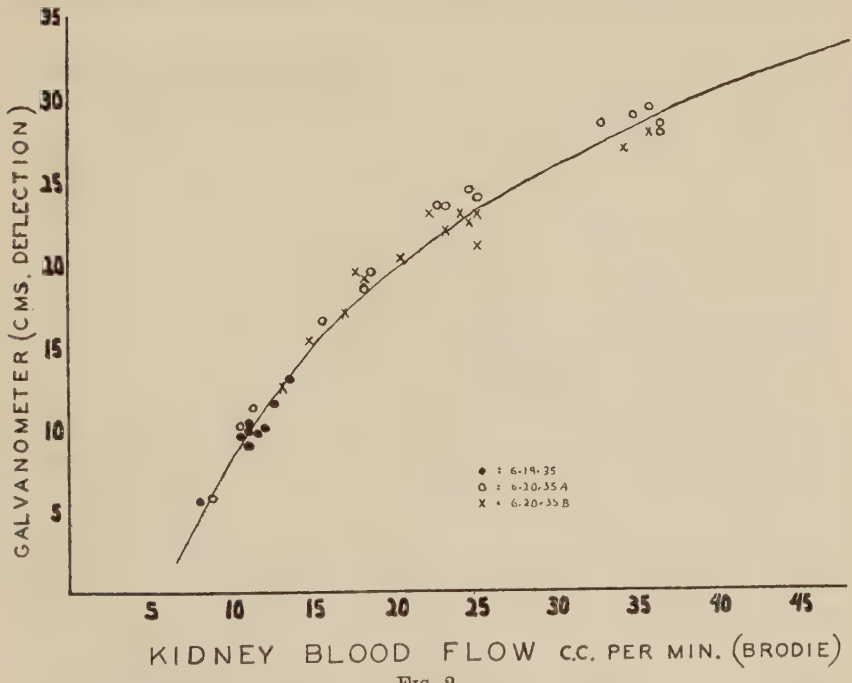


FIG. 2.

Acute calibration experiments. Unit 7-R-6 on renal veins of 3 rabbits. Form of curve: 1.0 cm. deflection of galvanometer equivalent to 7.72% change in blood flow. Heating current 2.0 amperes. Base line of galvanometer same in all experiments.

[2] Barcroft, J., Brodie, T. G., *J. Phys.*, 1905, **32**, 18.

the silver plate and the abdomen was closed, the base-line, (*i. e.*, the setting of the potentiometer, corresponding with the actual temperature difference between the two junctions) was constant for a given element and heating current applied to the same vessel of different animals of the same species; the position of the galvanometer image for a given renal blood-flow was also constant within approximately 10% except at flows lower than 10 cc. per min. Figs. 2 and 3 show representative groups of such experiments upon the renal veins and abdominal aortas, respectively, of rabbits. Similar results were obtained with the renal veins of 3 cats and the renal arteries of 5 dogs. Changes in abdominal temperature over the range 36-42°C. were without influence on the result. We have carried out corresponding calibrations upon the carotid, femoral, and iliac arteries of 3 dogs by perfusing the vessels with defibrinated blood by means of a pump; the curves were superimposable, but the base-lines varied considerably from animal to animal; we suspect that some of these discrepancies were due to adventitious circumstances, notably imperfections in the valves of the pump.

We have also performed calibration experiments upon animals in which an element had been installed upon the renal vein or aorta

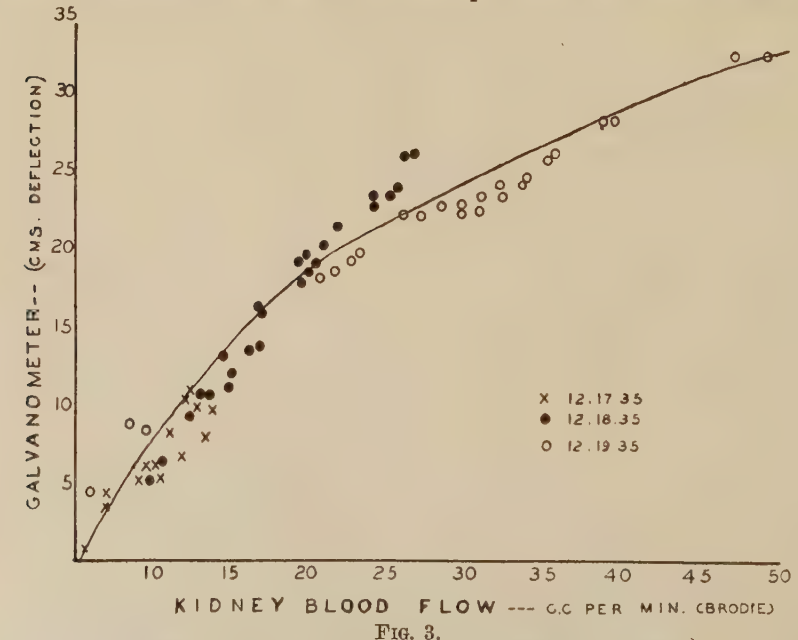


FIG. 3.

Acute calibration experiments. Unit 7-R-12 on abdominal aortas of 3 rabbits. Form of curve: 1.0 cm. deflection of galvanometer equivalent to an 8.33% change in blood flow. Heating current 2.0 amperes. Base line of galvanometer same in all experiments.

(rabbits) or renal artery (dogs) 24 hours previously. The aortic and renal arterial installations were successful: the base-line had not shifted appreciably and the calibration curves agreed with those obtained in acute experiments. The renal venous installations were unsuccessful because of shrinkage and distortion of the vein.

*Summary.* A thermostromuhr is described which differs principally from that of Rein in substituting storage battery for high frequency current. Its use is thereby vastly simplified. It has been calibrated on arteries and veins of living animals and gives promise of yielding quantitative information as to blood flow in unanesthetized animals.

We are anxious to express our gratitude to Dr. D. W. Bronk for his frequent helpful advice, and to Mr. A. J. Rawson of his department who has made for us the more perfect models of the instrument.

8368 C

## Effect of Adrenalin on Blood Sugar and Lactic Acid in Addison's Disease and in Adrenalectomized Dogs.

IAN ANDERSON.* (Introduced by G. A. Harrop.)

*From the Chemical Division of the Medical Clinic, Johns Hopkins Hospital, Baltimore.*

The hypothesis has recently been put forward that in adrenal insufficiency there is an inability of the muscles to produce lactic acid normally. Buell, Strauss and Andrus[1] found that in autolyzing pulverized skeletal muscle removed from cats suffering from experimental hyperthyroidism or adrenal insufficiency there was an inhibition of lactic acid formation, and suggested that the reason for the high blood lactate values found after exercise in patients suffering from hyperthyroidism or Addison's disease might be due to an impaired ability to metabolize the lactic acid formed rather than to an overproduction of lactic acid by the contracting muscles. Buell and Strauss[2] showed that in rats suffering from experimental hyperthyroidism the liver does not convert absorbed d-lactic acid into glycogen as readily as it does in normal animals, and the same

* Rockefeller Fellow.

1 Buell, M. V., Strauss, M. B., and Andrus, E. C., *J. Biol. Chem.*, 1932, **98**, 645.

2 Buell, M. V., and Strauss, M. B., *Bull. Johns Hopkins Hosp.*, 1934, **4**, 220.

has been shown to be true for rats suffering from chronic adrenal insufficiency (Buell, Strauss, and Anderson, unpublished data). It was considered of interest to find whether there was any similar disturbance of liver function (A) in patients suffering from Addison's disease, and (B) in dogs after double adrenalectomy.

(A) *Observations on patients with Addison's disease.* Two means of presenting d-lactic acid to the liver were considered—the injection of d-lactate, or the injection of adrenalin, which would cause a breakdown of muscle glycogen to lactic acid and raise the level of the blood lactic acid. The second method was chosen since it also allowed the response of the liver to an increase in the blood sugar to be observed. Cori[3] concluded that subcutaneous injection of adrenalin could be used with impunity in experiments to study the physiological effects of adrenalin, and this method was chosen in preference to continuous intravenous infusion. The dosage required to give a marked elevation of the blood lactic acid level was found to be 0.018 mg. of adrenalin per kilo body weight, and this dose was used in all the following experiments.

Three female patients suffering from Addison's disease were observed, 2 of whom (J. H. and F. B.) were being maintained on sodium chloride by mouth, while one (L. K.) required injections of adrenal cortical extract in addition, to maintain normal sodium and chloride levels in her blood. Three female patients, convalescent from lobar pneumonia, toxic arthritis, and malarial therapy for syphilis of the central nervous system, respectively, and of approximately the same weight as the patients with Addison's disease, were used as controls. The patients were all fasted for 12 hours before the experimental period and also during the experiment. A sample of blood was removed, and the appropriate dose of adrenalin injected subcutaneously. Further blood samples were taken 15, 30, 60, 90, 120, 180 and 240 minutes after the injection. The pulse rate and blood pressure were also taken at the same time as the blood samples. Blood lactic acid was determined by the method of Friedmann and Kendall[4] and blood glucose by the method of Hagedorn and Jensen.[5]

The results of individual experiments are shown in Table I. Since the individual results when plotted gave curves of approximately the same contours for each group, composite curves were constructed by averaging the individual values for each of the 2 groups (Fig. 1).

[3] Cori, C. F., *Physiological Reviews.*, 1931, **5**, 143.

[4] Friedmann, T. E., and Kendall, A. I., *J. Biol. Chem.*, 1929, **82**, 23.

[5] Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, **135**, 46.

TABLE I.
Time in Minutes After Adrenalin Injection.

| (1) Normals | | 0 | 15 | 30 | 60 | 90 | 120 | 180 | 240 |
|---|---|---|---|---|---|---|---|---|---|
| 1. R.L. | Blood glucose | 106 | 154 | 165 | 171 | 160 | 131 | 115 | 92 |
| | " lactate | 21 | 69 | 48 | 21 | 23 | 23 | 16 | 12 |
| | Pulse rate | 66 | 82 | 78 | 76 | 74 | 70 | 68 | 70 |
| | Blood pressure | 140/88 | 104/58 | 112/68 | 116/80 | 104/72 | 108/66 | 106/68 | 110/78 |
| 2. B.E. | Blood glucose | — | 178 | 208 | 205 | 149 | 114 | 143 | 99 |
| | " lactate | 30 | 50 | 66 | 61 | 36 | 36 | 25 | 24 |
| | Pulse rate | 76 | 84 | 98 | 108 | 112 | 108 | 94 | 94 |
| | Blood pressure | 106/68 | 148/98 | 130/80 | 112/60 | 94/64 | 98/64 | 96/62 | 92/62 |
| 3. F.H. | Blood glucose | 88 | 127 | 167 | 203 | 194 | 158 | 124 | 88 |
| | " lactate | 10 | 23 | 32 | 38 | 28 | 29 | 14 | 19 |
| | Pulse rate | 62 | 62 | 74 | 80 | 86 | 80 | 76 | 78 |
| | Blood pressure | 106/68 | 174/68 | 170/68 | 114/60 | 112/64 | 108/64 | 104/64 | 106/58 |
| (2) Addison's Disease | | | | | | | | | |
| 1. L.K. | Blood glucose | 89 | 139 | 161 | 186 | 164 | 117 | 81 | 73 |
| | " lactate | 9 | 30 | 31 | 31 | 34 | 34 | 17 | 17 |
| | Pulse rate | 66 | 68 | 90 | 94 | 92 | 86 | 72 | 70 |
| | Blood pressure | 124/86 | 188/90 | 150/82 | 108/66 | 120/74 | 114/72 | 114/78 | 108/74 |
| 2. F.B. | Blood glucose | 93 | 128 | 130 | 186 | 150 | 152 | 123 | 97 |
| | " lactate | 9 | 17 | 26 | 25 | 27 | 25 | 21 | 15 |
| | Pulse rate | 78 | 78 | 82 | 82 | 84 | 74 | 84 | 86 |
| | Blood pressure | 86/54 | 110/78 | 114/78 | 100/66 | 92/66 | 110/74 | 90/62 | 86/60 |
| 3. J.H. | Blood glucose | 66 | 107 | 133 | 142 | 180 | 149 | 100 | 66 |
| | " lactate | 22 | 36 | 45 | — | 40 | 47 | — | 26 |
| | Pulse rate | 74 | 76 | 80 | 80 | 84 | 80 | 88 | 86 |
| | Blood pressure | 92/56 | 106/66 | 96/52 | 98/56 | 88/52 | 90/60 | 82/58 | 86/58 |

The blood glucose and blood lactate values are in milligrams per 100 cc. blood.

The normal individuals showed a blood lactate curve which rises rapidly, reaching a maximum within 30 minutes after the injection of adrenalin and falling more slowly to reach the basal level within 3 hours. In the patients with Addison's disease, the blood lactate curve, starting from a lower basal level, rose almost as sharply as in normal individuals, but instead of falling rapidly, remained elevated and did not return to the basal level in 4 hours. The absolute increase in blood lactate was slightly less than in normal individuals, 20 mg. % as against 27 mg. %.

The blood sugar curves showed approximately the same contour in the 2 groups, reaching a maximal level within one hour and falling

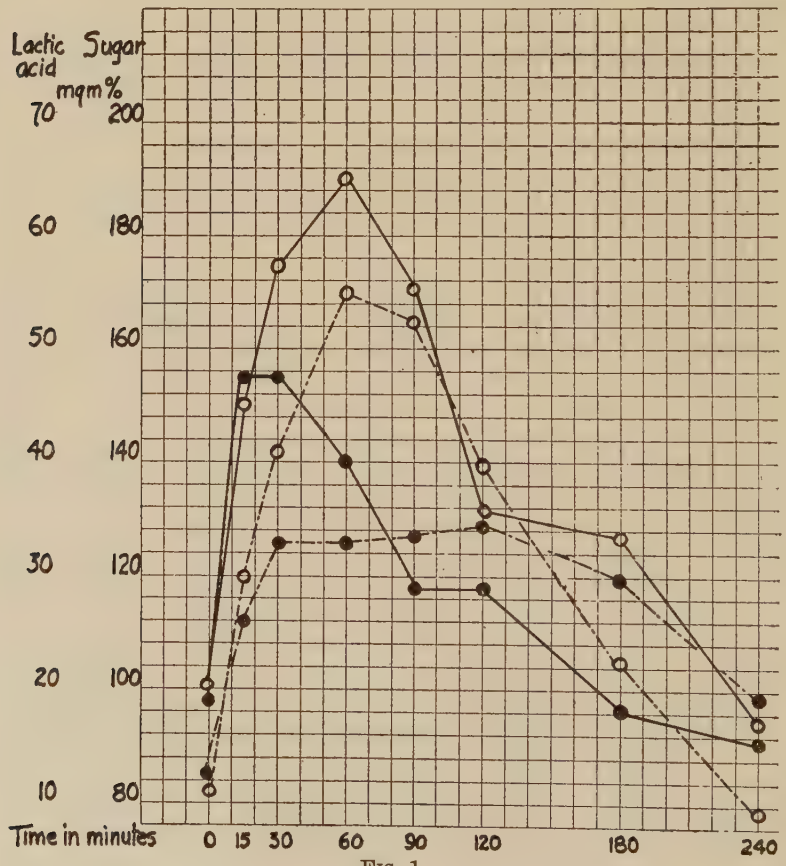


FIG. 1.

Blood sugar and blood lactate curves following subcutaneous injection of adrenalin—0.018 mg. per kilo body weight in normal individuals and in patients with Addison's Disease—composite curves (three cases in each group).

Sugar—normal o——o
Lactic acid—normal ●——●
Sugar—Addison's o- - - - -o
Lactic acid—Addison's ●- - - - -●

to basal level in 4 hours. The initial basal level was significantly lower in the patients with Addison's disease, 83 mg. % as against 97 mg. %, but the absolute increase showed little difference: 88 mg. % as against 96 mg. % in the normals.

The continued elevation of the blood lactate curve in the patients with Addison's disease was not due to slower absorption of adrenalin from the subcutaneous tissues owing to low blood pressure, since the blood sugar curve did not show a corresponding contour; also 2 of the normal controls had blood pressures within the range of those found in the patients with Addison's disease. It was noted that in the 2 cases of Addison's disease with hypotension (F. B. and J. H.) the increase in pulse rate and blood pressure was markedly less than in the 2 normal controls (F. H. and B. E.) who also had hypotension and who received approximately the same total amount of adrenalin. This would appear to indicate that in Addison's disease there is a lessened sensitivity to the cardio-accelerator and pressor effects of adrenalin.

It would appear that in patients suffering from Addison's disease who are kept in approximately normal health by means of sodium chloride or sodium chloride plus adrenal cortical extract, adrenalin can cause a conversion of muscle glycogen to lactic acid as readily as in normal individuals. The delay in the disappearance of the accumulated lactate from the blood seen in such patients may be due to a sluggishness on the part of the liver to convert lactic acid into glycogen, as was found in rats suffering from chronic adrenal insufficiency.

The power of the liver to synthesize glycogen from blood sugar is evidently not impaired, as the disappearance of the blood glucose formed from liver glycogen under the action of adrenalin was just as rapid in the cases of Addison's disease as in the normal individuals.

B. *Observations on adrenalectomized dogs.* The effect of adrenalin on the blood sugar and blood lactate of adrenalectomized dogs was studied by 2 experiments on the same animals, one before and the second after the removal of the second adrenal gland in the 2-stage operation for bilateral adrenalectomy, it being assumed that animals with one intact adrenal gland would give a normal response to adrenalin injection, since both their general condition and the concentration of arterial plasma electrolytes were normal. After the second gland had been removed, the dogs were allowed to develop adrenal insufficiency to make sure that both glands had been completely removed, and were then restored to approximately normal

condition by the administration of adrenal cortical extract as described by Harrop *et al.*[6] The second adrenalin experiment was not performed until the animals were in good condition as judged by their weight and the chemical findings in the heparinized arterial plasma.

It was decided to give the adrenalin as a single intravenous injection, since this would shorten the experimental period during which the animals had to be kept as quiet as possible, although it was recognized from the work of Markowitz *et al.*[7] that the injected adrenalin would be rapidly destroyed in the tissues. The dose of adrenalin required to produce a marked elevation of blood lactate was found to be 0.036 mg. per kilo body weight. The dogs were accustomed to being fastened down on a table, and at the beginning of the experimental period, previous to which no food had been given for 12 hours, they were tied down, and a sample of blood removed from the jugular vein, after which the appropriate dose of adrenalin was injected, and blood samples removed after 5, 15, 30, 45, 60 and 90 minutes, the dog being returned to its cage during the intervals between the taking of the last 3 samples.

Two such experiments have been performed, the results of one of which are shown in Fig. 2. The blood lactate curve in the control experiment (single adrenalectomy) showed the same rapid rise as was found in the human controls, but the descent of the curve was more prolonged and the basal level had not been reached in 90 minutes. After bilateral adrenalectomy, the blood lactate curve showed a flattening of its contour, the increase in blood lactate being only 13 mg. % as against 26 mg. % in the control experiment. It also showed a tendency towards a prolongation of the elevated level, though this was not nearly so striking as in the patients with Addison's disease.

The blood sugar curve after bilateral adrenalectomy showed a slower rise to the maximal level than in the control experiment, and a flattening of the contour of the curve, the increase in blood sugar being only 32 mg. % as against 51 mg. % in the control experiment. In neither case had the basal level been regained in 90 minutes, but the descent of the curve was slightly slower in the animal after bilateral adrenalectomy, only 13 mg. % of blood sugar disappearing in the last hour of the experiment as against 37 mg. % in the control. This affords some evidence of delay in the removal of glucose

[6] Harrop, G. A., and Weinstein, A., *J. Exp. Med.*, 1933, **57**, 305.

[7] Markowitz, J., and Mann, F. C., *Am. J. Physiol.*, 1929, **89**, 176.

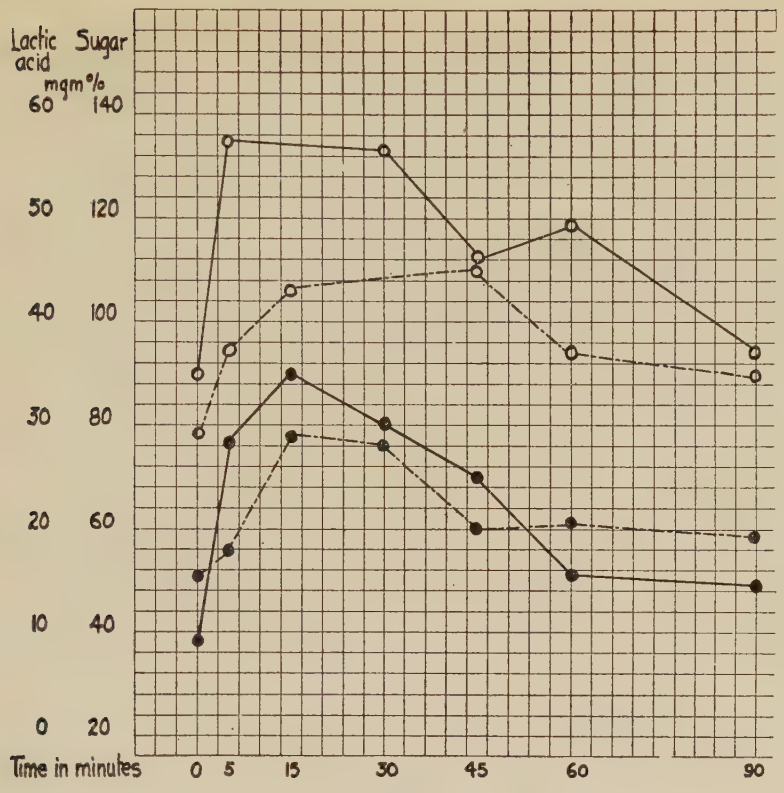


FIG. 2.

Blood sugar and blood lactate curves following intravenous injection of adrenalin (0.036 mg. per kilo body weight) in a dog after (1) unilateral adrenalectomy, and (2) bilateral adrenalectomy.

Unilateral—blood sugar o——o Bilateral—blood sugar o - - - - o
adrenalectomy—blood lactate ●——● adrenalectomy—blood lactate ● - - - - ●

from the blood stream, as was noted by Fernandez *et al.*[8] in dogs 48 hours after bilateral adrenalectomy, in which 2 gm. of glucose per kilo body weight were injected intravenously.

The second experiment gave substantially similar results.

*Summary.* 1. The effect of adrenalin on the blood sugar and blood lactate was studied: A. in patients with Addison's disease. B. in adrenalectomized dogs. 2. In patients with Addison's disease, the subcutaneous injection of adrenalin produced a similar increase in the blood sugar to that observed in the controls, and the blood sugar curve showed approximately the same contour in the 2 groups. The blood lactate curves differed, however. Both showed a similar

[8] Fernandez, R., Foglia, V. G., Leloir, L. F., and Novelli, A., *Compt. rend. soc. Biol.*, 1934, **115**, 334.

sharp rise, but in the patients with Addison's disease, the curve remained elevated, and had not returned to basal level in 4 hours, whereas in the normal controls, it had reached basal level again within 3 hours. 3. It is suggested that the delay in the disappearance of the accumulated lactate from the blood seen in patients with Addison's disease may be due to a sluggishness on the part of the liver to convert lactic acid to glycogen. 4. The blood sugar and blood lactate curves, following a single intravenous injection of adrenalin in adrenalectomized dogs, are both lower and tend to remain elevated longer than in the same dogs before removal of the second adrenal gland.

## 8369 C

### Suspension Stability of Erythrocytes in Solutions of Globulin.*

S. P. LUCIA, S. M. GOSPE AND J. W. BROWN.

*From the Departments of Medicine, and Obstetrics and Gynecology, University of California Medical School, San Francisco.*

Fahraeus[1] showed that the most rapid sedimentation of erythrocytes took place in solutions of fibrinogen, less rapid in solutions of globulin, and least rapid in solutions of albumin. He concluded that the rapidity of sedimentation and the increased percentage of serum globulin and fibrinogen occurring coincidentally "stand in direct causality."[2] In confirmation of the thesis of Fahraeus, Westergren[3, 4] obtained the following coefficients of correlation between the *sedimentation rate* and fibrin, globulin, and albumin:

$$r_{SR\text{-}F} + 0.82,\ r_{SR\text{-}G} + 0.50,\ r_{SR\text{-}A} - 0.46, \text{ and } r_{SR\text{-}FGA} + 0.87$$

Using a modification of the Linzenmeier[5] technique, Lucia and coworkers[6] obtained a correlation coefficient of —0.27 between *sedi-*

*Assisted by a grant from the Christine Breon Fund.

1 Fahraeus, R., *Acta. Med. Scand.*, 1921, **55**, 1.

2 Fahraeus, R., *Physiol. Rev.*, 1929, **9**, 255.

3 Westergren, A., Theorell, H., and Widstrom, G., *Z. f. d. g. Exp. Med.*, 1931, **75**, 668.

4 Westergren, A., Juhlin-Dannfelt, C., and Schnell, R., *Acta. Med. Scand.*, 1932, **77**, 469.

5 Linzenmeier, G., *Arch. f. Gynaekologie*, 1920, **113**, 608.

6 Lucia, S. P., Blumberg, T., Brown, J. W., and Gospe, S. M., to be published.

*mentation time* and serum globulin.† They concluded that a cause and effect relationship cannot be said to exist between these factors. This hypothesis was put to trial by testing the effects of various solutions of serum globulin *in vitro* on the sedimentation time of erythrocytes.

All sedimentation experiments were done using the Friedlander tube and recording the time necessary for the column of erythrocytes to settle 18 mm. A 20 cc. sample of venous blood was withdrawn and oxalated. A sedimentation test was done directly on this sample, using the Linzenmeier technique. The remainder of the sample was separated by centrifugalization and the corpuscular moiety washed with 3 changes of Locke's solution. Then 0.2 cc. of washed corpuscles were resuspended in 0.8 cc. of the following menstrua: plasma, Locke's solution, and dilutions of globulin in plasma and in Locke's solution. The resulting cell volumes were 20% or $2.15 \pm 0.15$ million corpuscles per cmm. The globulins were prepared by electrodialysis.‡ In all resuspension experiments the syringes were rinsed in 10% potassium oxalate solution.

In 3 experiments in which increasing concentrations of beef blood pseudoglobulin was suspended in Locke's solution, the sedimentation time was prolonged beyond that of the control. The same phenomenon was observed in 2 experiments in which beef blood globulin was used.

In 2 experiments in which a liquid solution of human blood pseudoglobulin was diluted with Locke's solution, the sedimentation time was also prolonged beyond that of the control. In 7 experiments in which powdered globulin from the same source, and ranging in concentration from 1 to 5% in Locke's solution, produced a progressive diminution of the sedimentation time when compared with the control. There were 4 experiments in which this same powdered globulin was dissolved in blood plasma and in which the sedimentation time was prolonged beyond that of the control.

In 5 experiments in which a faintly bile-tinged solution of globulin prepared from human ascitic fluid and ranging in concentrations up to 12% in Locke's solution was used, there was noted a progressive diminution in the sedimentation time. In 5 experiments a powdered globulin, from the same source, diluted in Locke's solution markedly prolonged the sedimentation time. This latter

† A negative correlation between sedimentation time and globulin is similar to a positive correlation between sedimentation rate and globulin.

‡ Courtesy of Dr. D. M. Greenberg of the Department of Biochemistry.

product acted similarly, but to a lesser degree, in 4 experiments in which it was dissolved in plasma.

*Summary.* An analysis of the effects of 6 different types of globulin, dissolved in Locke's solution, upon the sedimentation time of erythrocytes, reveals that in 12 experiments 4 of the types prolonged the sedimentation time, and that in 12 experiments the remaining 2 types shortened the sedimentation time. In 8 experiments in which 2 of the types of globulin were dissolved in plasma, the sedimentation time was prolonged.

*Conclusion.* These experiments indicate that a consistent relationship cannot be established *in vitro* between the sedimentation time of erythrocytes and the globulin content of various solutions of serum globulin.

8370 P

## A Comparison of the Tar Tumors of Rabbits and the Virus-Induced Tumors.

PEYTON ROUS AND J. W. BEARD.

*From the Rockefeller Institute for Medical Research, New York City.*

By tarring the ears of domestic and cottontail rabbits tumors have been procured for comparison with others experimentally induced with the Shope virus.[1] The tarred rabbits were strictly isolated.

The growths caused by virus regularly developed from the surface epidermis and were papillomas varying little in structure, whereas not a few of those consequent on tarring originated from the skin appendages, with result in a wider morphological variety. The tar papillomas were scattered, discrete and often punctate in origin; so too were the papillomas due to virus when this had been appropriately inoculated. The growths due to tarring appeared only after it had been repeated often enough to cause general hyperplasia of the epithelium, together with complex connective tissue alterations; and many of them retrogressed after tarring was stopped. The virus tumors, on the other hand, arose on the basis of the slight epidermal trauma incident to inoculation, and their progression was followed, not preceded, by connective tissue changes. The virus evidently needed no help,—though the growths it caused could be stimulated to rapid enlargement by connective tissue disturbances experimentally

[1] Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

induced.[2] The unknown cause of the tar papillomas, on the other hand, was effective only when epithelial and connective-tissue disturbances had long existed, becoming independent thereof only after the growths had undergone considerable development, and often not even then. The tar papillomas were structurally much more irregular than early virus papillomas, as would follow from the disturbance of the underlying connective tissue, which was of a sort to bring about corresponding irregularities in the virus tumors. The surface of the tar papillomas often underwent maceration because covered, whereas the exposed surface of the virus growths consisted of dry, keratinized material.

When due allowance had been made for these differing conditions of incidence and growth, the resemblance between the tar and virus papillomas was found to be close. Often growths of the 2 kinds could not be told apart under the microscope except by the diffuse hyperplasia consequent on tarring. This was notably true of tumors induced in cottontail rabbits, the natural hosts of the virus. In general, however, the tar papillomas were less pigmented or unpigmented, as would follow from the fact that they originate from a single cell or from but few.[3] Melanoblasts are only passively included in the epidermal papillomata, with rare exceptions[4]; and the fewer the cells from which these arise the less likely are such elements to be included in quantity with a resulting pigmentation of the growth. This holds strikingly true of the virus papillomas, which, when punctate in origin, are often wholly unpigmented even when originating from a deeply pigmented skin.

Sometimes the granular epithelial layer was relatively pronounced in the tar papillomas and the cells became flatter before they keratinized, features referable to a slower differentiation. Retrogression of growths of both kinds took place in the same way.[5]

Cottontails possess considerable resistance to the virus-induced papillomas, as evidenced by their slow enlargement and frequent retrogression. They have never in our experience gone on to cancer, whereas the especially vigorous growths of domestic rabbits have frequently done so.[6] The changes taking place then are identical with those occurring when a tar-induced papilloma becomes a carcinoma; but in the latter instance the transformation is often more

2 Beard, J. W., and Rous, Peyton, *J. Exp. Med.*, 1934, **60**, 723.

3 Mottram, J. C., *J. Path. and Bact.*, 1935, **40**, 407.

4 Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1334.

5 Beard, J. W., and Rous, Peyton, *J. Exp. Med.*, 1934, **60**, 723.

6 Rous, Peyton, and Beard, J. W., *J. Exp. Med.*, 1935, **62**, 523.

rapid, as one would expect from the favoring local conditions. Whether such conditions will cause the virus papillomas of cottontails to become cancerous remains to be seen. Frequently the alteration of a virus papilloma to a squamous cell cancer halts for a time at one stage or another, and in consequence cystic tumors or malignant papillomas develop, growths unusual after tarring.

In one cottontail small virus papillomas grew for a while, at 2 of 3 inoculation sites on the trunk. Fourteen weeks after their disappearance tarring of the ears was begun. One growth reappeared during the 5½ months of tarring, and a new papilloma developed at the site that had previously been negative. The new tumor progressed after tarring was stopped, but the growth which had recurred disappeared once more. In untarred rabbits a reappearance of retrogressed papillomas due to virus has never been observed. The fact is attested, however, that intercurrent stimulation may influence decisively the course of established growths.[7]

To summarize, the results of the comparison accord with the conception that the tar tumors may be due to a virus or viruses ensconsed in the epidermis and dependent for pathogenic activity upon tissue derangements such as are produced by tar.

8371 C

## A Convenient Method for the Preparation of Concentrates of Follicle Stimulating Hormone from Urine.*

E. BRAND, R. J. BLOCK, M. M. HARRIS AND L. E. HINSIE.

*From the Departments of Chemistry, Internal Medicine and Clinical Psychiatry, New York State Psychiatric Institute and Hospital, New York.*

In the course of investigations on the excretion of gonad stimulating hormones in the urine of mental patients[1, 2] the following method was developed for the preparation of concentrates of the follicle stimulating hormone (F.S.H.).

[7] Kidd, John G., Rous, Peyton, and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 193.

* This investigation was aided in part by a grant from the Board of Directors of the Neuro-Psychiatric Institute of the Hartford Retreat, Hartford, Conn. We are indebted to Dr. C. Charles Burlingame, Psychiatrist-in-Chief of the Institute, for his continued interest in this problem.

[1] Harris, M. M., Brand, E., and Hinsie, L. E., *Am. J. Psychiat.*, 1935, **91**, 1239.

[2] Harris, M. M., Brand, E., Hinsie, L. E., and Block, R. J., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1576.

The method is based on the observation that the stable aluminum hydroxide C ($\gamma$), prepared according to Willstaetter and Kraut[3] is a satisfactory absorbent for F.S.H. from urine. The effectiveness of this adsorption depends markedly on the pH, a definite maximum occurring at pH 4.5. Evans, Gustus and Simpson[4] have reported that solutions of the gonadotropic hormone from acetone-dried pregnant mare's serum are more satisfactorily adsorbed at pH 3.5 by aluminum hydroxides of Types A and B, prepared according to an earlier method of Willstaetter and Kraut,[5] while Type C was considerably less efficient.

The urine is collected in glass jars containing 5 cc. of a saturated alcoholic solution of thymol per liter capacity. Insoluble material is removed by filtration and the filtrate is adjusted to exactly pH 4.5 with 5 N HCl using bromcresol green as external indicator. A suspension containing 20 gm. per liter of aluminum hydroxide C ($\gamma$) is slowly poured into the urine until 1 gm. of aluminum hydroxide per liter of urine has been added. For 24-hour specimens of 500-1000 cc. a full 1.0 gm. of aluminum hydroxide is added; for samples of less than 500 cc. only 0.5 gm. is used. The resulting mixture is occasionally stirred by hand (mechanical stirring results in poor yields) during 20 minutes so as to maintain a homogeneous suspension; the solids are allowed to settle during 45 minutes. The supernatant fluid is removed by siphoning and centrifugation, readjusted to pH 4.5 and again treated with aluminum hydroxide by the same procedure. The combined precipitate is centrifuged at high speed in 100 or 250 cc. vessels, washed with 10 volumes of acetone, and transferred with acetone to 35 or 50 cc. centrifuge tubes in which the acetone washing is repeated until dehydration is complete.† The product is dried *in vacuo* over calcium chloride, pulverized and stored in the refrigerator.

For the elution of the F.S.H. the dry powder, weighing 3-6 gm. for an average 24-hour specimen, is suspended in 7 cc. of 0.01 N NaOH and the pH adjusted to 10-10.5 by the careful addition of

[3] Willstaetter, R., and Kraut, H., *Ber. Deut. Chem. Ges.*, 1923, **56**, 1117; 1924, **57**, 1082. For nomenclature *cf.* Kraut, H., in Oppenheimer, C., *die Fermente*, Leipzig, 1929, **3**, 480.

[4] Evans, H. M., Gustus, E. L., and Simpson, M. E., *J. Exp. Med.*, 1933, **58**, 569.

[5] Willstaetter, R., and Kraut, H., *Ber. Deut. chem. Ges.*, 1923, **56**, 150.

† The dehydration with acetone permits the storage of the activated aluminum hydroxide and also removes any theelin which might have been carried down in the precipitation. Dehydration, however, is not essential and elution of the wet precipitate yields satisfactory results.

5 N NaOH.‡ The mixture is thoroughly stirred and centrifuged. If at this point the pH of the supernatant liquid is found to be below 10, it is raised to this level by the addition of more 5 N NaOH, stirred and again centrifuged. After decanting the supernatant fluid, the solid is treated with 0.01 N NaOH to give a volume of 7-8 cc. and the pH again brought to 10-10.5. The process is repeated until the supernatant liquid is practically colorless; the combined extracts, amounting to 20-27 cc., are neutralized§ to pH 7.4 with 5 N HCl and employed for biological assay.|| If a more concentrated solution is required, the hormone may be precipitated by pouring the extract into 10 volumes of cold acetone, dried *in vacuo* and redissolved. Any insoluble material may be removed by centrifuging, for it is inactive.

The above processes can be satisfactorily carried out on larger volumes of urine. After elution the neutralized extract may, if desired, be decolorized with charcoal (1-2 gm. of Carbex E per 100 cc. of extract) at room temperature without apparent loss of activity. By acetone precipitation of such extracts a slightly colored, hygroscopic powder is obtained in yields of 8-9 gm. per 100 gm. of aluminum hydroxide. This material contains about 50% of ash and 2.7-3.5% of nitrogen (uncorrected). Qualitative tests on the precipitates indicate little or no organic sulfur and no reducing substances either before or after acid hydrolysis. A positive Sakaguchi reaction has repeatedly been obtained. In the standard rat test 60 mg. of such preparations produce ovaries weighing 50-60 mg. A large amount of such a homogeneous preparation is being used at present in an attempt to establish a unit of the follicle stimulating hormone suitable for clinical purposes.

Concentrates prepared by the aluminum hydroxide method are nontoxic when injected even in large amounts into immature female rats, in contrast to samples prepared by precipitation with ethyl alcohol.

Comparison of concentrates prepared by the above process and that involving benzoic acid[6] indicates that aluminum hydroxide is superior for the concentration of the follicle stimulating hormone

‡ Elution of the dry powder with ammonia, $Ba(OH)_2$, $Ca(OH)_2$ or pyridine has proved to be unsatisfactory.

§ Sometimes a precipitate is formed at this point, which is apparently inactive and should be removed by centrifugation.

|| The assay on immature female rats is carried out by the usual technique (*cf.* 2).

[6] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1934, **106**, 125.

while the benzoic acid procedure gives better results with the luteinizing hormone,** when these methods are applied to urine. Our experiments indicate that the aluminum hydroxide method leads to a recovery of at least 60% of the F.S.H. originally present in the urine.

The above described method has been satisfactorily used for daily determinations of the amount of F.S.H. excreted in the urine of more than 150 patients suffering from various mental and nervous diseases. Various physiological and clinical aspects of the findings will be discussed in a detailed report which is being prepared for publication in the *Psychiatric Quarterly*.

The investigations are being continued.

## 8372 C

### Influence of Ultra Violet Irradiation on Clam Heart Subjected to Potassium Excess.

S. A. GUTTMAN. (Introduced by H. S. Liddell.)

*From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y., and Marine Biological Laboratory, Woods Hole, Mass.*

This investigation was undertaken in an attempt to determine the effect of ultra violet on tissue subjected to potassium excess. For this purpose the heart of the clam, *Venus mercenaria,* was used.

Records were obtained of the ventricular beat of the heart *in situ.* A small hook, made of glass capillary tubing, was inserted into the apex of the ventricle and, by means of a thread attached to the hook and a light balanced lever (a drinking straw), records were obtained on a moving kymograph. The clam, on the half shell, was placed in a finger bowl which was filled with sea water. To this the desired amount of potassium chloride was added.

The ultra violet source was a Cooper-Hewitt Uviarc (6-inch tube with reflector and running at 110 volts D. C.) placed at 35 cm. from the preparation. A thermopile, with a blackened couple, at the heart, served as a temperature index. The sensitivity of the galvanometer enabled temperature changes of 0.01°C. to be observed. During all of the experiments the temperature changes were negligible. Room temperature varied from 22 to 27°C. During the course of an experiment the temperature both of the room and of the heart

** Antuitrin-S was used as the source of L.H. We are indebted to Dr. Oliver Kamm of Parke, Davis and Co., for considerable quantities of this material.

never changed more than one degree Centigrade from the beginning of the experiment to the end. Temperature rise during irradiation never exceeded 0.3°C.

Short ultra violet irradiation (2-3 min.) of the clam heart appears to cause a marked increase in tonus, a definite decrease in amplitude, but does not affect the pace maker.

Experiments were performed with potassium chloride concentrations up to 2.7% in sea water. When concentrations above 1.36% potassium chloride were used ultra violet (1-2 min.) did not always restore a rhythm. This was the case in 3 experiments out of 20. The preparations were then thoroughly washed in sea water. Neither this nor mechanical stimulation was effective in restoring a rhythm. When the foot was stimulated mechanically it did not respond. It is probable that in these 3 experiments the tissue had been killed by excess potassium. This leads me to believe that approximately the maximum concentration of potassium was employed during the course of this investigation and that ultra violet irradiation is effective in overcoming potassium which is present in these concentrations. The amounts of potassium were so great that the solutions employed were hypertonic. This hypertonicity caused a marked increase in tonus and ultra violet was also able to overcome this. Thus it appears that ultra violet irradiation may alter the potassium-calcium equilibrium.

Lieber[1] studied, by micro-chemical methods, the distribution of potassium and calcium in the skin of white rats and guinea pigs and found a potassium excess in the hair follicles and in the depths of the epidermis. Following X-ray treatment he reports that the potassium in the vicinity of the hair follicles practically disappears. Calcium was found to be redistributed to occupy the region where the potassium excess originally appeared, *i. e.*, the follicles and depths of epidermis. Adler and Wiederhold[2] reported partial disappearance of potassium from blood serum which was X-rayed. Numerous investigators, Lepeschkin[3] on blood erythrocytes, Lepeschkin,[4] Blackman and Paine,[5] and Tröndle[6] on plant cells, have reported an increase in permeability after ultra violet treatment. It appears that somehow an increase in permeability occurs and pos-

[1] Lieber, G. D., *Strahlentherapie*, 1925, **20**, 93.
[2] Adler, K., and Wiederhold, O., *Strahlentherapie*, 1932, **44**, 383.
[3] Lepeschkin, W. W., *Protoplasma*, 1933, **18**, 243.
[4] Lepeschkin, W. W., *Am. J. Bot.*, 1930, **17**, 953.
[5] Blackman, V., and Paine, S. G., *Ann. Bot.*, 1918, **32**, 69.
[6] Tröndle, A., *Jahrb. wiss. Bot.*, 1910, **48**, 171.

sibly a shift in potassium-calcium equilibrium takes place. The potassium is enabled to leave the cell and calcium may go in and thus enable a normal equilibrium to be attained. It is quite possible that this physical shift is, in part, able to account for some of the biological effects of ultra violet.

## 8373 C

### Notes on the Weil-Felix Reaction in Individuals not Suffering from Typhus.

SADASHIVARAO R. SAVOOR, M. RUIZ CASTANEDA AND HANS ZINSSER.

*From the Department of Bacteriology and Immunology, Harvard University Medical School.*

In view of the increasing frequency with which the Weil-Felix reaction is being used in the United States for the diagnosis of obscure fevers suspected of Rickettsia origin, and especially because of our own interest in the possibility of latent typhus infection in individuals infected a long time ago, we undertook to carry out a considerable number of Weil-Felix reactions on individuals not at the time suffering from typhus or fevers of any kind, for the purpose of establishing the significance of low titre reactions. We are reporting these briefly because they seem to us helpful in appraising occasional doubtful cases.

The reactions were carried out on sera from several groups of subjects. One group was composed of Jewish out-patients furnished us by the Beth Israel Hospital Clinic in Boston, and in these record was made as to whether the individual was born in Russia or in the United States. Since such information had no significant effect on these observations, we abstain from tabulating it. Another

TABLE I.
Weil-Felix Reactions.

| Material | Total No. | Positive Reactions 1-20 | 1-40 | 1-80 | 1-160 | % of Positives 1-20 or over % |
|---|---|---|---|---|---|---|
| Jewish patients, Beth Israel Hospital | 123 | 7 | 10 | 7 | 1 | 20+ |
| Non-Jewish patients | 242 | 18 | 19 | 10 | 1 | 19+ |
| Routine Wassermann sera | 207 | 25 | 18 | 6 | 2 | 24+ |
| Russian-born garment workers | 24 | 0 | 2 | 2 | 0 | 17+ |
| Totals | 596 | 50 | 49 | 25 | 4 | 21+ |

group was composed of non-Jewish subjects, another of routine Wassermann sera in which no attention was paid to the race of the patient; and, finally, a small group of 24 Russian-born garment workers. Table I gives the results.

It will be seen that there was no significant difference between the groups, and it is rather fortunate in protecting us against false conclusions that the actual percentage of positive reactions in the Russian-born garment workers was rather lower than in the other groups.

In the Jewish group, 13.9% had reactions of 1-40 or below.

In the non-Jewish group, 15.3% had reactions of 1-40 or below.

In the routine Wassermann group, 20.7% had reactions of 1-40 or below.

There were 25 of the 596 cases with reactions of 1-80.

There were only 4 cases in which the reactions went up to 160. The follow-up on these cases gave no significant information.

The obvious conclusion is that a Weil-Felix reaction of 1-40 or below usually has no significance and reactions of 1-80 can be observed without necessarily pointing to Rickettsia infection.

The study has no bearing on our opinion that Brill's disease, or imported European typhus fever, represents a recrudescence of an infection acquired many years ago and held latent in the bodies of a small percentage of those who get over the disease, for the blood Weil-Felix usually disappears within a few months in convalescents. One would not, therefore, expect a Weil-Felix reaction in people who have had typhus fever years ago, unless they were actually suffering from a recrudescence or unless the agglutinating power for the Proteus X-19 reappeared non-specifically under the influence of some febrile condition.

8374 C

## Paradoxic Streptococcus Antiserums.*

EDWARD E. DART. (Introduced by W. H. Manwaring.)

*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.*

If a stock culture of *Streptococcus hemolyticus* is grown on 5% rabbit blood-agar plus 10% commercial antistreptococcus serum, the hemolytic zone surrounding each plate colony is at times 100% to 200% greater than the control area of hemolysis on routine blood-agar plus 10% normal horse serum. A typical example of this auxolytic effect is recorded in Table I (serum A).

TABLE I.

Parallel antihemolytic and auxohemolytic tests with 3 typical commercial streptococcus antiserums.† The table records average areas of hemolysis per colony on 48-hour blood-agar plates (37.5° C.).

| Streptococcus X grown on 5% blood-agar plus: | Hemolytic area (aver.) | Relative % |
|---|---|---|
| 10% normal horse serum (control) | 89 sq.m. | 100 |
| 10% commercial antiserum A | 167 " | 188 |
| 10% " " B | 66.4 " | 75 |
| 10% " " C | 15.6 " | 18 |

† The normal and commercial antistreptococcus serums used in these and other tests were kindly furnished by Eli Lilly and Co., The Cutter Laboratory, Lederle Laboratories, E. R. Squibb and Sons, and Parke, Davis and Co.

The percentage increase in average hemolytic area varies (a) with the concentration of the auxolytic serum tested and (b) with the selected strain of *S. hemolyticus*. Typical variations are recorded in Table II.

TABLE II.

Streptococcus A, kindly furnished by the manufacturer, was the main strain used in the production of commercial antistreptococcus serum A. Both antiserum and normal serum in this table were free from chemical preservatives.

| | Aver. area of hemolysis per colony with: | | | | | |
|---|---|---|---|---|---|---|
| | Streptococcus Y | | | Streptococcus A | | |
| Parallel hemolysis on 5% blood-agar plus: | Normal horse serum sq.mm. | Anti-serum A sq.mm. | Relative % | Normal horse serum sq.mm. | Anti-serum A sq.mm. | Relative % |
| 1% horse serum | 21.5 | 54.6 | 254 | 5.37 | 6.92 | 129 |
| 10% " " | 35.6 | 75.7 | 216 | 5.37 | 6.28 | 116 |
| 50% " " | 75.7 | 107.2 | 142 | 8.3 | 1.89 | 23 |

The above recorded auxohemolytic effects are reminiscent of the

* Work supported in part by the Rockefeller Fluid Research Fund of Stanford Medical School.

paradoxic growth-acceleration of certain bacteria in the presence of homologous specific immune serum.[1]

## 8375 C

## Evidence of Permeability of Tissue Cells to Potassium.

J. I. THALER. (Introduced by W. O. Fenn.)

*From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester.*

It is often stated, most recently by Hastings and Eichelberger,[1] that tissue cells are impermeable to potassium. Evidence to the contrary has been presented by Fenn and Cobb[2] from experiments on frogs. This paper reports certain experiments on cats leading also to the conclusion that the cells are permeable to potassium.

If successive blood samples are withdrawn over a period of 3 to 4 hours from the common carotid artery of a cat, and analyzed for potassium* a steady rise in potassium content of the plasma is ob-

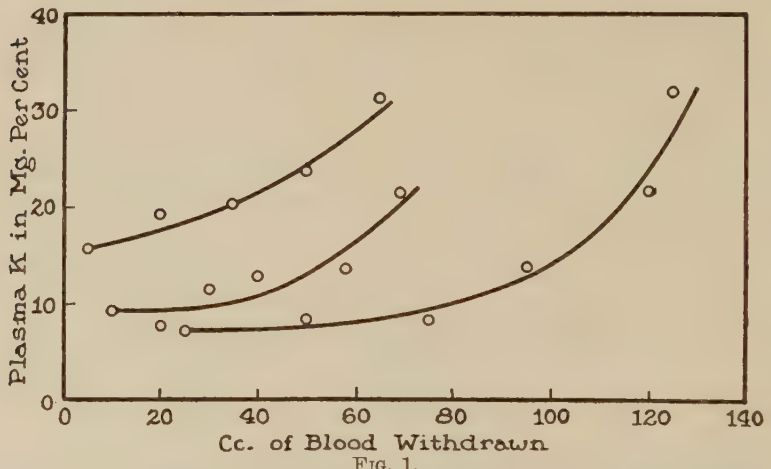


FIG. 1.

Results of 3 experiments showing the progressive rise in plasma potassium as successive samples of 10-25 cc. of blood were withdrawn for analysis.

[1] Nicolle, M., and Césair, E., *Ann. Inst. Pasteur*, 1926, **40**, 43.

[1] Hastings, A. B., and Eichelberger, L., *J. Biol. Chem.*, 1935, **109**, xli.

[2] Fenn, W. O., and Cobb, D. M., *J. Gen. Physiol.*, 1934, **17**, 629; *Am. J. Physiol.*, 1935, **112**, 41.

* The blood was dry-ashed in a muffle furnace at 500°C. and analyzed by the method of Shohl and Bennett (*J. Biol. Chem.*, 1928, **78**, 643) except that the precipitate of potassium was separated by centrifuge.

served as indicated by the graphs of Fig. 1. In this way 40 to 50% of the total blood volume can be withdrawn, bringing the potassium concentration to approximately 30 mg. % before death ensues. Since the completion of these experiments a similar result has been published by Baetjer.[3] She found further that any procedure which reduced the circulation by 80% of its normal value caused a similar rise in plasma potassium. This potassium apparently comes from the tissue cells after the tissue spaces have given up to the blood all the fluid they can spare, but the physico-chemical mechanism is not clear. If the cells are normally impermeable to potassium it might be regarded as the result of an injury to the cells accompanied by an increase of permeability. That this is not correct is indicated, however, by experiments in which blood or saline was reinjected after the potassium had been increased by a previous hemorrhage, with the result that the plasma potassium returned to normal. *i. e., potassium diffused back into the cells.* Were it assumed that there is a return of permeability to a normal condition of impermeability to potassium, this reversal would be impossible. Data from 2 of 4 similar experiments of this sort are shown in Table I. The figures

TABLE I.
Effect of Blood and Saline Injection on Plasma Potassium.

| Time | Blood drawn | r.b.c. | Whole blood | Plasma | r.b.c. |
|---|---|---|---|---|---|
| min. | A. Ureters not ligated. Wt. of cat = 2.8 Kg. | | | | |
| | cc. | % | mg. potassium per 100 cc. | | |
| 0 | 10 | 54.1 | 12.9 | 10.4 | 15.0 |
| 65 | 30 | (incubated at 37° C.) | | | |
| 95 | 10 | 37.5 | 16.0 | 16.4 | 15.2 |
| 110 | 30 | (reinjected intravenously) | | | |
| 130 | 10 | 43.2 | 11.7 | 9.0 | 15.3 |
| | B. Ureters ligated. Wt. of cat = 3.2 Kg. | | | | |
| 0 | 10 | 35.3 | 14.6 | 11.0 | 21.2 |
| 35 | 40 | (drawn and discarded) | | | |
| 75 | 10 | 35.0 | 17.5 | 15.4 | 21.4 |
| 95 | 40 | (normal saline injected intravenously) | | | |
| 120 | 10 | 28.5 | 13.5 | 10.6 | 20.7 |

The animals were under Dial-urethane anesthesia.

show the results of analyses made on successive samples of blood, together with the time of bleeding and the amount drawn.

In the first animal (A) the plasma potassium was increased from 10.4 to 16.4 mg. % after removal of 40 cc., but returned to 9.0 mg. % after reinjection of 30 cc. of blood. In the second animal (B), the ureters were ligated to prevent loss of potassium into the

[3] Baetjer, A. M., *Am. J. Physiol.*, 1934, **109**, 3.

bladder. After raising the plasma potassium from 11.0 to 15.4 mg. % by removal of 50 cc. of blood, 40 cc. of saline were injected to bring up the blood volume, and the plasma potassium decreased to 10.6 mg. %. In the last column, the potassium content of the cells is calculated showing that the potassium content of the cells did not change, *i. e.*, the whole-blood potassium shows similar changes.

These potassium changes cannot, therefore, be ascribed to the blood cells, to the kidneys, or to the tissue spaces which must contain potassium at nearly the same concentration as the plasma. Some tissue cells must be concerned. It seems simplest to assume, therefore, that these cells are normally permeable to potassium and that shifts of potassium are the result of disturbances in the physico-chemical membrane equilibrium such as might be produced by an acid-base change either in the blood or the tissues, or both.

Another attempt has been made to drive an excess of potassium into the tissue cells. With the ureters ligated to eliminate loss through the kidneys, 45 cc. of 0.2 M KCl were injected intravenously; 45 minutes after completion of injection, the plasma potassium had increased from an initial value of 13.1 mg. % to 61 mg. %, while the corresponding values for striated muscle were 422.4 mg. % and 426.6 mg. %, respectively. This small increase in the muscle can readily be accounted for by an increased concentration of potassium in the tissue spaces parallel to the increase in the plasma. Even if the cells are permeable to potassium, the potassium must enter either by exchange with hydrogen ion (or some other cation), or as undissociated potassium hydroxide. It cannot enter with another anion since muscles are apparently anion-impermeable. Any entrance of potassium into the cells is therefore probably limited by the alkalinity which results and was imperceptible under the conditions of this experiment.

Another procedure which increases the blood potassium is injection of histamine. In one experiment the plasma potassium rose from an initial concentration of 10.8 mg. % to 16.5 mg. % after an intravenous injection of 0.05 mg. % of histamine per kilo of cat.

D'Silva[4] has also reported an increase in plasma potassium after hemorrhage. He suggested that the rise is not due solely to stimulation of the sympathetics resulting in a secretion of epinephrine, for while the first effect of injection of epinephrine is an increase of potassium in the blood this rise is all over in 5 minutes. My experiments also show that 5 minutes after injection of 1/10,000

[4] D'Silva, J. L., *J. Physiol.*, 1933, **80**, 7P; 1934, **82**, 393.

epinephrine no significant change in plasma potassium can be observed.

*Conclusions.* The concentration of potassium in the plasma increases as the circulatory volume decreases. This is a reversible process since the potassium concentration returns to normal if the circulatory volume is increased subsequently by reinjection. Saline is as effective as whole blood in causing the decrease of potassium concentration in this way. The tissue cells are reversibly permeable to potassium ions. The injection of epinephrine has only a transitory effect on the plasma potassium but histamine causes a more lasting increase.

I am very grateful to Dr. W. O. Fenn for his aid with the experiments and in the preparation of this manuscript.

8376 C

## On the Absence of Thiolhistidine in Insulin.

VINCENT DU VIGNEAUD, ROBERT H. SIFFERD AND GAIL MILLER.

*From the Department of Biochemistry, School of Medicine, The George Washington University, Washington, D. C.*

Considerable evidence has pointed to the possibility of the presence in insulin of sulfur other than that attributable to cystine, in which form the major portion of the sulfur is present. That thiolhistidine might account for part of this sulfur was a possibility to be considered. We therefore undertook studies to ascertain whether or not this amino acid is a constituent of the insulin molecule. On the basis of reports in the literature that the sulfur of thiolimidazoles could be split off as sulfate by boiling with ferric chloride and that the sulfur of cysteine was stable, we attempted to test for the presence of thiolhistidine. However, in testing this reaction with cystine, we found that ferric chloride will oxidize the sulfur of cystine to sulfate.[1] This reaction could not, therefore, be utilized to detect thiolhistidine in the presence of cystine in an insulin hydrolysate and some other method of approach was necessary.

In Barger and Ewin's early paper on the betaine of thiolhistidine, ergothioneine it was shown that the sulfur of this compound was

[1] Sifferd, R. H., and du Vigneaud, V., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 332.

oxidized by bromine to inorganic sulfate.[2] In addition, it is a well recognized fact that cystine upon treatment with bromine is converted to cysteic acid and that no inorganic sulfate is formed.[3] It occurred to us that these 2 observations might form the basis of the desired test but before applying this reaction to insulin for the detection of thiolhistidine we felt that it was necessary to test the reaction on the amino acid itself rather than depend on the known behavior of its betaine. This amino acid was, therefore, synthesized by Harington's method,[4] using aspartic acid as starting material. We were able to check Harington's procedure in all essential details and obtained approximately the same yields reported in the various steps recorded by him. Upon treatment of the synthetic thiolhistidine with bromine the sulfur was converted into sulfate. As was to be expected, cystine yielded no sulfate under the same conditions. The same was true of glutathione, methionine, and homocystine. Furthermore we found that zein, in which the presence of thiolhistidine had been suspected,[5] did yield sulfate after similar treatment with bromine. Recently the bromine oxidation of thiolimidazole as a test for this type of sulfur in protein has been independently developed by Blumenthal and Clarke[6] and applied with excellent results to a series of proteins. In addition to zein they found that other proteins, particularly keratin, yielded sulfate by this method of oxidation.

After the feasibility of this method of approach was established, the reaction was applied to crystalline insulin. Five hundred mg. of the insulin was hydrolyzed by refluxing it with 50 cc. of 20% HCl for 8 hours. The sample was evaporated to dryness, the residue was taken up in 10 cc. of water and bromine water was added until a slight excess of bromine was present. The test for sulfate ion with barium chloride was negative. Knowing that the sulfur in hydrolyzed insulin was mainly if not all in the disulfide state and thinking that this might make some difference in the behavior of a thiolimidazole which would conceivably be present in a disulfide form, we reduced a sample of hydrolyzed insulin and then treated it with bromine. Likewise no sulfate was obtained. Finally the reaction was applied to the unhydrolyzed insulin and again as in the previous tests no sulfate could be detected.

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[2] Barger, G., and Ewins, A. J., *J. Chem. Soc.*, 1911, **99**, 2336.

[3] Andrews, J. C., *J. Biol. Chem.*, 1933, **102**, 263.

[4] Harington, C. R., and Overhof, J., *Biochem. J.*, 1933, **27**, 338.

[5] Eagles, B. A., and Vars, H. M., *J. Biol. Chem.*, 1928, **80**, 615.

[6] Blumenthal, D., and Clarke, H. T., *J. Biol. Chem.*, 1935, **110**, 343.

On the basis of these results we feel that the conclusion is justified that thiolhistidine is not present in the insulin molecule.

## 8377 C

### Resistance of the Spider Monkey (Ateles ater) to Infection with the Virus of Acute Anterior Poliomyelitis.

EATON M. MACKAY AND CHARLES R. SCHROEDER.

*From the Research Laboratory, San Diego Zoological Society, San Diego, and The Scripps Metabolic Clinic, La Jolla, California.*

Monkeys are the only animals definitely known to be susceptible to poliomyelitis and generally speaking, varieties of old world monkeys seem more susceptible[1, 2] than those of the western hemisphere. Of the new world monkeys members of the genus *Cebus* (ringtail) have been thoroughly investigated. Although transmission of the experimental disease to a *Cebus* monkey has been reported in one instance[1] this South American species can now be considered to be completely refractory.[2, 3, 4] Although not an inexpensive species, the spider monkey like the ringtail can be obtained with relative ease on the Pacific Coast and the shortage of *Macacus rhesus* specimens for titering serum in the 1934 poliomyelitis epidemic in Los Angeles made it desirable to reexamine the susceptibility of the spider.

We are indebted to Doctor Simon Flexner for *Macacus rhesus* cord infected with his well-known mixed (M. V.) virus. This was used as a 10% suspension in buffered saline. Inoculations were made intracerebrally in the usual manner. Typical symptoms and paralyses occurred in all of the *Macacus rhesus* specimens which were inoculated. When no symptoms had developed in the spider monkeys after 10 days a second or accelerating dose of 1 cc. of the suspension was injected.[5, 6] One spider monkey was observed for 30 days after the first injection and the other 2 for 60 days without showing symptoms. At the end of 60 days one was killed and a

[1] Flexner, S., and Lewis, P. A., *J. Exp. Med.*, 1910, **12**, 227.

[2] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, **54**, 45.

[3] Kraus, R., and Kantor, L., *Rev. d. Inst. Bact.*, 1917, **1**, 43.

[4] Jungeblut, C. W., and Engle, E. T., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 879.

[5] Flexner, S., *Science*, 1931, **74**, 520.

[6] Flexner, S., *Science*, 1933, **77**, 413.

brain suspension of the injected area injected intracerebrally into a *Macacus rhesus*. This monkey did not develop the disease.

TABLE I.

| No. | Species | Sex | Weight Kg. | Volume Suspension cc. | Max. Temp. F. | Paralysis days | Death days |
|---|---|---|---|---|---|---|---|
| 1 | *Macacus Rhesus* | ♀ | 2.8 | 1.00 | 104.0° | 5 | 7 |
| 2 | " " | ♀ | 3.8 | 0.75 | 105.8° | 9 | 10 |
| 3 | " " | ♀ | 3.5 | 0.50 | 105.2° | 6 | 6 |
| 4 | " " | ♂ | 2.9 | 0.10 | 106.1° | 6 | 9 |
| 5 | *Ateles ater* | ♀ | 3.3 | 0.50 | 100.4° | Second dose 1 cc. virus suspension 10 days after first. | |
| 6 | " " | ♂ | 3.6 | 1.00 | 101.6° | Second dose 1 cc. virus suspension 10 days after first. | |
| 7 | " " | ♀ | 4.1 | 1.50 | 102.8° | Second dose 1 cc. virus suspension 10 days after first. | |

*Conclusions.* The spider monkey, *Ateles ater,* like other new world varieties, is naturally refractory to experimental inoculation with poliomyelitis virus (monkey passage).

## 8378 C

### Albumin-Globulin Ratios in Synthetic Solutions from Specific Gravity and Relative Viscosity Measurements.

R. L. NUGENT AND L. W. TOWLE. (Introduced by Stuart Mudd.)

*From the Department of Chemistry, the University of Arizona, Tucson.*

Nugent and Towle[1] have reported specific gravity values for 33 solutions of beef serum albumin, serum globulin and mixtures of the 2 in the range from 0 to 12% total protein. In all cases the solution contained 0.9% sodium chloride and was adjusted to pH 7.3 to 7.5. Under these conditions, the specific gravity of a solution was shown to be a measure of its total protein content in accord with the finding of Moore and Van Slyke[2] that the specific gravity is a useful measure of total plasma protein in nephritis, more useful in fact than the refractive index, the physical property which has been most widely used in this connection.

At the same time relative viscosity values were obtained for a sim-

[1] Nugent, R. L., and Towle, L. W., *J. Biol. Chem.*, 1934, **104**, 395.

[2] Moore, N. S., and Van Slyke, D. D., *J. Clin. Inv.*, 1930, **8**, 337.

ilar series of solutions which have not previously been reported. The solutions for the relative viscosity measurements were prepared and their specific gravity, salt content and pH value controlled in the same manner as previously described. The relative viscosity measurements were made with a 1 cc. Ostwald viscometer at 25° in a water bath thermostat. Since the relative rates of flow of water and protein solutions through capillary tubes may vary with the applied pressure[3] the values obtained may well be specific for an instrument of the dimensions employed. An instrument of convenient dimensions was therefore indicated since it must serve as a model for others to be employed in checking or utilizing the results. The one chosen for use* required a small volume of solution in accord with the amounts of serum usually available in routine clinical work, and was of a type which could be reproduced by an amateur glass blower. (Fig. 1.) The upper and lower bulbs contain respectively 0.6 and 0.9 cc. and their centers are 70 mm. apart. The capillary tube is 67 mm. long and 0.50 mm. in diameter. At 25° using a total

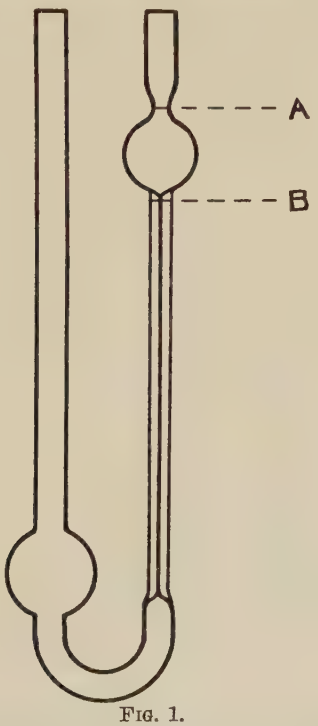


FIG. 1.
An Ostwald viscometer of the type employed.

[3] Kruyt, H. R., (translated by van Klooster, H. F.), New York, 1930, 182.

* A type which has been used by S. DeW. Ludlum and his associates.

volume of 1.0 cc. of distilled water, the time required for the level to drop from A to B is 36.7 seconds as measured with a stopwatch. The tubing for the rest of the instrument has an internal diameter of 3 mm. The relative viscosity of a protein solution is given by the equation, relative viscosity $= T_p \times d_p/36.7$, where $T_p$ is the time required for the level of the protein solution to drop from A to B, and $d_p$ is the specific gravity of the protein solution.

The relative viscosity values are plotted against the total protein percentages in Fig. 2, which shows that smooth curves are obtained for each of the 6 albumin-globulin ratios, varying regularly from

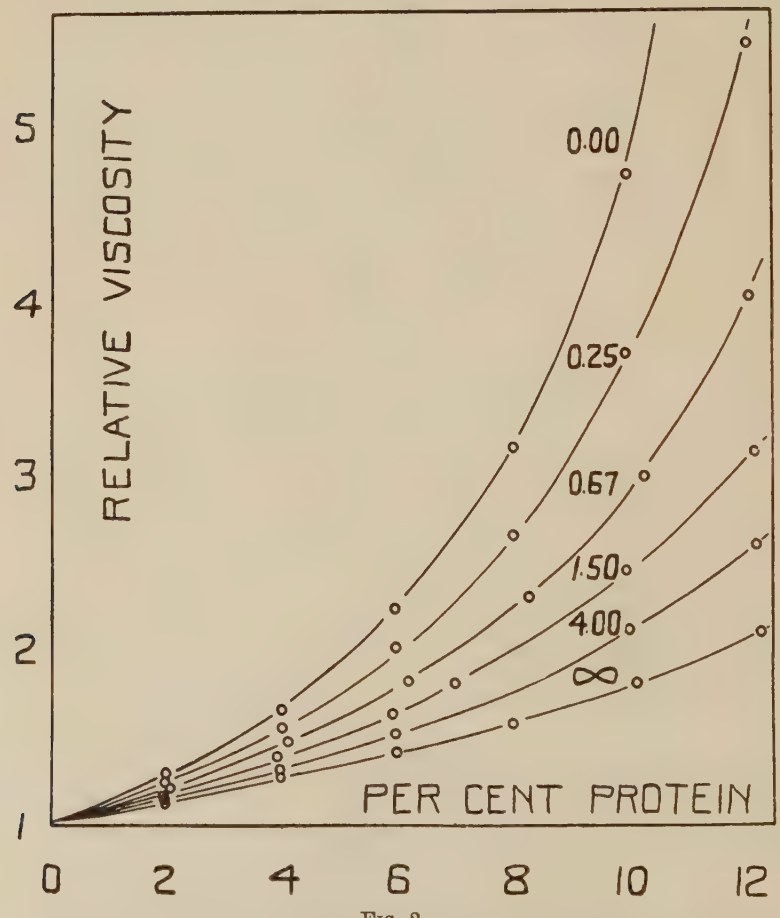


FIG. 2.

The relative viscosity values for synthetic solutions of beef serum albumin and serum globulin plotted against the corresponding specific gravities. The number to the left of each curve indicates the albumin-globulin ratio of the solutions represented by that curve.

the very slightly convex curve for 100% albumin to the markedly convex curve for 100% globulin.[4]

It is apparent that having determined the total protein content by means of a specific gravity measurement it should be possible to estimate the albumin-globulin ratio by determining the relative viscosity and plotting the point on the diagram. The method is entirely analogous to the refractive index-relative viscosity method of Heyder,[5] Rohrer[6] and Bircher.[7] However, the salt contents and pH values of the synthetic solutions employed by these workers were not carefully controlled, and they did not point out the possibility of the advantageous use of a simple standard viscometer of the Ostwald type. In addition, as has been mentioned, there is reason to believe that the specific gravity of plasma is a better measure of total protein than the refractive index. The application of the method described here to blood serum was originally suggested by Ludlum, Taft and Nugent.[8]

In testing the method, solutions were prepared by one of the authors and analyzed by the other with results as shown in Table I.

With the exception of solution No. 2, which was apparently subject to gross experimental error, the total percentages agree to within about one part in a hundred. Eight of the nine albumin-globulin ratios obtained are in satisfactory agreement with the actual values.

TABLE I.

Comparison of Actual Total Protein Values and Albumin-Globulin Ratios of a Series of Mixed Solutions of Serum Albumin and Serum Globulin with Those Determined by the Specific Gravity-Relative Viscosity Method

| Solution No. | Actual total protein % | Total protein found % | Actual albumin-globulin ratio | Albumin-globulin ratio found |
|---|---|---|---|---|
| 1 | 10.00 | 10.00 | 0.11 | 0.09 |
| 2 | 7.00 | 7.14 | 0.11 | 0.08 |
| 3 | 3.50 | 3.55 | 0.11 | 0.09 |
| 4 | 9.55 | 9.53 | 1.00 | 0.89 |
| 5 | 7.00 | 7.03 | 1.00 | 1.00 |
| 6 | 4.50 | 4.53 | 1.00 | 0.89 |
| 7 | 10.00 | 10.00 | 9.00 | 9.00 |
| 8 | 7.00 | 6.96 | 9.00 | 7.33 |
| 9 | 4.50 | 4.44 | 9.00 | 10.11 |

[4] Lloyd, D. J., Chemistry of the Proteins, Philadelphia, 1926, 160.

[5] Heyder, E., Estimation of the Refractivity and Viscosity of Globulin and Albumin Solutions and Their Mixtures, Tubingen, 1915.

[6] Rohrer, F., *Deutsch. Arch. f. klin. Med.*, 1916, **121**, 221.

[7] Bircher, M. E., *J. Lab. Clin. Med.*, 1921, **7**, 134.

[8] Ludlum, S. DeW., Taft, A. E., and Nugent, R. L., unpublished discussion.

In general, the results indicate that the method is useful with synthetic solutions under controlled conditions. Its accurate application to blood sera would require in any case corrections for effects of varying concentrations of sodium chloride, glucose and urea upon both the specific gravity and relative viscosity values and of possible abnormal serum proteins upon the relative viscosity in various pathological conditions.

## 8379 C

### Ultrafiltration of the Virus of Equine Encephalomyelitis.

JOHANNES H. BAUER, HERALD R. COX AND PETER K. OLITSKY.

*From the Laboratories of the International Health Division of the Rockefeller Foundation, and the Rockefeller Institute for Medical Research, New York.*

Meyer, Haring, and Howitt[1] were the first to show that the virus of equine encephalomyelitis passes readily through Berkefeld V and N filters. Olitsky, Cox and Syverton[2] reported that the virus passed through Seitz filters in a relatively high concentration. Krueger, Howitt and Zeilor[3] filtered the virus through acetic acid collodion membranes and found that it passed through 3%, but was retained by 3.5% membranes. From these results they estimated the particle size of the virus to be approximately 500 mμ. It was considered of interest to determine the size of this virus more accurately by filtration through finely graded collodion membranes of relatively uniform porosity.

The collodion membranes used in our experiments were prepared according to the method of Elford[4] with certain modifications adopted by Bauer and Hughes.[5] The filtration technique described by Bauer and Hughes[6] in their study of yellow fever virus was closely followed. All filtrations were conducted under positive pressure of nitrogen of 100 cm. Hg. The effective filtration area of the membranes comprised about 5 cm.$^2$ and the amount of filtrate collected through such an area varied from 8 to 10 cc.

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1 Meyer, K. F., Haring, C. M., and Howitt, B., *Science*, 1931, **74**, 227.
2 Olitsky, P. K., Cox, H. R., and Syverton, J. T., *J. Exp. Med.*, 1934, **59**, 159.
3 Krueger, A. P., Howitt, B., and Zeilor, V., *Science*, 1933, **77**, 288.
4 Elford, W. J., *J. Path. and Bact.*, 1931, **34**, 505.
5 Bauer, J. H., and Hughes, T. P., *J. Gen. Physiol.*, 1934, **18**, 143.
6 Bauer, J. H., and Hughes, T. P., *Am. J. Hyg.*, 1935, **21**, 101.

The Eastern and Western strains of the virus were studied. These were the same strains that had previously been employed by Cox and Olitsky[7] in their immunization experiments with the virus adsorbed on aluminum hydroxide. Brains of mice that succumbed to experimental infection were used as source of virus, and in one experiment the virus grown in tissue cultures was employed. In preparing material for filtration, 5 to 8 brains were finely ground in a sterile mortar and suspended in a diluent made up of equal parts of hormone broth, ascitic fluid and distilled water. The suspension was centrifuged in the angle centrifuge for 30 minutes at a speed of 3,000 r.p.m., and the supernatant fluid was passed through a Seitz filter. Portions of this stock filtrate were passed through a series of collodion membranes of varying porosities. With a view to reducing the extent of adsorption of the virus by the membranes, 4 cc. of broth were passed through each membrane prior to the filtration of the virus. When tissue culture material was used as a source of virus, there were added to 60 cc. of tissue culture 20 cc. each of hormone broth, ascitic fluid, and distilled water. The mixture was then centrifuged and the supernatant fluid passed through a collodion membrane with an average pore diameter of 600 mμ to serve as stock filtrate. The presence of the virus in the filtrates was determined by injection of the filtrates intracerebrally into mice; 4 to 6 mice were used for each filtrate and each mouse was given 0.03 cc. In each experiment the virus content of the stock filtrate was titrated in mice using 4 to 6 mice for each dilution.

TABLE I.
Filtration Experiment with Mouse-Brain Virus, Western Strain.

| No. of Membrane | Aver. pore diameter mμ | Amt. of filtrate collected cc. | Results of inoculation of filtrate in mice* | Titration of stock filtrate | |
|---|---|---|---|---|---|
| | | | | Dilution | Results* |
| 207 | 75 | 9 | 5/6 | $10^{-2}$ | 6/6 |
| 222 | 70 | 9 | 4/6 | $10^{-3}$ | 6/6 |
| 139 | 66 | 9 | 2/6 | $10^{-4}$ | 4/6 |
| 208 | 60 | 8 | 0/6 | $10^{-5}$ | 0/6 |
| 176 | 55 | 8 | 0/6 | | |

*In all the tables the numerator represents the number of mice that succumbed to infection; the denominator, the number of mice used in the test.

In Table I are summarized the results of one of the 4 identical filtration experiments carried out with the Western strain of the mouse-brain virus. The virus passed through membranes with an average pore diameter of 75, 70, and 66mμ, but was held back by

[7] Cox, H. R., and Olitsky, P. K., *Science*, 1934, **79**, 459.

those of 60 and 55 mμ. The results of the experiments carried out with the Eastern strain, using both mouse-brain and tissue culture virus are shown in Tables II and III. The results were exactly the same as those obtained with the Western strain.

TABLE II.
Filtration Experiment with Mouse-Brain Virus, Eastern Strain.

| No. of Membrane | Aver. pore diameter mμ | Amt. of filtrate collected cc. | Results of inoculation of filtrate in mice | Titration of stock filtrate | |
|---|---|---|---|---|---|
| | | | | Dilution | Results |
| 207 | 75 | 10.0 | 6/6 | 10–2 | 4/4 |
| 139 | 66 | 10.0 | 6/6 | 10–3 | 4/4 |
| 208 | 60 | 8.5 | 0/6 | 10–4 | 4/4 |
| 176 | 55 | 8.0 | 0/6 | 10–5 | 0/4 |
| 202 | 50 | 7.5 | 0/6 | | |
| 150 | 45 | 7.0 | 0/6 | | |

TABLE III.
Filtration Experiment with Tissue Culture Virus, Eastern Strain

| No. of Membrane | Aver. pore diameter mμ | Amt. of filtrate collected cc. | Results of inoculation of filtrate in mice | Titration of stock filtrate | |
|---|---|---|---|---|---|
| | | | | Dilution | Results |
| 207 | 75 | 10 | 6/6 | 10–2 | 6/6 |
| 222 | 70 | 10 | 5/6 | 10–3 | 6/6 |
| 139 | 66 | 10 | 5/6 | 10–4 | 6/6 |
| 208 | 60 | 10 | 0/6 | 10–5 | 4/6 |
| 176 | 55 | 10 | 0/6 | | |

Bauer and Hughes[6] have shown that yellow fever virus passes through collodion membranes with pore diameters close to the filtration end-point in a relatively high concentration, indicating a remarkable uniformity in the size of both the virus particles and the pores of membranes. A similar experiment was carried out with the Eastern strain of mouse-brain virus, in which the virus content of the filtrates of different membranes was titrated in mice. All dilutions were made in a diluent consisting of equal parts of ascitic fluid, broth, and distilled water, as used in the preparation of the stock filtrate. The results are shown in Table IV. It was rather surprising to find that although the filtration end-point was sharp and clear-cut, the virus appeared in the filtrates in a fairly low concentration, proving infective only in 1-100 dilution. It will be noticed also that 75% of the protein present in the stock filtrate had been adsorbed or held back by the membranes. As to whether the low concentration of the virus in the filtrates was caused by the lack of uniformity in the particle size of the virus, or the pore size of the membranes, or by the reduction of the size of the pores in the

TABLE IV.
Filtration Experiment with Mouse-Brain Virus, Eastern Strain. Titration of filtrates.

| No. of Membrane | Aver. pore diameter mμ | Amt. of filtrate collected cc. | Protein content of filtrate % | Results in mice inoculated with dilutions of the filtrates | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Undiluted | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ |
| Seitz | | | 0.2 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 5/6 |
| 207 | 75 | 8.0 | 0.05 | 6/6 | 5/6 | 6/6 | 0/6 | 0/6 | 1/6* |
| 222 | 70 | 8.0 | 0.05 | 6/6 | 6/6 | 2/6 | 0/6 | 0/6 | 0/6 |
| 139 | 66 | 8.5 | 0.05 | 6/6 | 6/6 | 3/6 | 0/6 | 0/6 | 0/6 |
| 208 | 60 | 8.0 | 0.05 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| 176 | 55 | 8.0 | 0.05 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |

*The cause of death uncertain; probably not encephalitis.

membranes through the adsorption of a large amount of protein, is impossible to determine.

*Summary.* The virus of equine encephalomyelitis, both the Eastern and the Western strains, was found to pass collodion membranes with an average pore diameter of 66 mμ, but was completely held back by those of 60 mμ. The results indicate that this virus has the same filtration end-point, and consequently the same particle size, as that of St. Louis encephalitis, which was shown by Bauer, Fite, and Webster,[8] and Elford and Perdrau[9] to be 20 to 30mμ.

## 8380 C

### Staphylococcal Antihemolysin in Osteomyelitis and Other Staphylococcal Infections.

JOHN E. BLAIR AND FRANCES A. HALLMAN.

*From the Laboratory Division, Hospital for Joint Diseases, New York City.*

The possible clinical application of staphylococcal toxoid in staphylococcal infections makes it necessary to know the amount of circulating antitoxin in the blood of normal individuals, and of persons with staphylococcal infections before treatment with toxoid.

Several recent reports[1–6] have recorded antihemolysin (antitoxin?) values for normal healthy individuals. The earlier reports are not readily comparable because of differences in standards employed in various laboratories. The general conclusion, however, is that sera from normal persons exhibit a wide individual variation of titers. With the establishment of a provisional International Standard for staphylococcus antitoxin,[7] it is possible to render reports in entirely comparable terms. Dolman[3] has reported that the normal value for healthy individuals is from ⅓ to 1 International Unit. Murray[5]

[8] Bauer, J. H., Fite, G. L., and Webster, L. T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 696.

[9] Elford, W. J., and Perdrau, J. R., *J. Path. and Bact.*, 1935, **40**, 143.

[1] Bryce, L. M., and Burnet, F. M., *J. Path. and Bact.*, 1932, **35**, 183.

[2] Dolman, C. E., *J. Am. Med. Assn.*, 1933, **100**, 1007.

[3] Dolman, C. E., *Lancet*, 1935, **1**, 306.

[4] Parish, H. J., O'Meara, R. A. Q., and Clark, W. H. M., *Lancet*, 1934, **1**, 1054.

[5] Murray, D. S., *Lancet*, 1935, **1**, 303.

[6] Gross, H., *Klin. Wchnschr.*, 1933, **12**, 907.

[7] Hartley, P., and Llewellyn Smith, M., *Quart. Bull. Health Organis., League of Nations*, 1935, Jan., 68.

reported the normal values as ranging from 0.4-0.7 to 2 International Units.

The titration of antihemolysin in staphylococcal infections has, for the most part, been done upon sera of patients with superficial infections, although some sera from cases of osteomyelitis have also been tested. Dolman[2] reported that patients with superficial infections "show no more, and often less, circulating antitoxin than do healthy persons of the same age." In contrast, Connor and McKie[8] found that "patients with superficial staphylococcal infections have in most cases higher titres of antihaemolysin than normal persons." Parish, O'Meara, and Clark,[4] in a few tests of sera from superficial infections, found the same variation in range of titer as in sera from normal persons. The titers obtained by Murray[5] in superficial infections covered the same general range as did the normal sera, with a slightly larger number of higher titers in the group of infections. Murray considered that the difference was not sufficiently great to be of significance.

The general opinion is recorded in the literature that sera from cases of osteomyelitis possess antihemolysin titers definitely above the normal range. Thus, Dolman[3] records titers of 5-6, and occasionally of 10 International Units. Murray[5] records titers averaging 11.7 International Units, and states "there seems general agreement that in osteomyelitis alone is the amount greatly increased."

We have been interested particularly in the staphylococcal antihemolysin content of sera from cases of osteomyelitis, with reference to subsequent treatment of patients with staphylococcal toxoid. This communication records staphylococcal antihemolysin values for a group of patients with osteomyelitis, and for a group of controls, consisting of normal individuals and persons with staphylococcal infections other than osteomyelitis.

The antihemolysin titrations were originally done by determining that dilution of serum which just inhibited hemolysis of 1% rabbit erythrocytes by one M.H.D. of staphylococcus toxin, after incubation for 1 hour at 37°C. Subsequently, a sample of the International Standard Antitoxin was obtained through the courtesy of Dr. Hartley, and our own sera were titrated in terms of the standard. The titers recorded in this paper are in terms of the International Unit.

The toxin used was prepared from the "Ha" strain of *Staphylo-*

[8] Connor, J. I., and McKie, M., *J. Path. and Bact.*, 1933, **37**, 353.

*coccus aureus,* isolated by Dr. Burky. Our culture was obtained through the courtesy of Dr. Roberts, of the Lederle Laboratories. Cultures were grown in semisolid (0.3%) agar, in an atmosphere of 30% $CO_2$, for 48 hours at 37°C. The culture was then drained through gauze, and filtered through a Seitz filter. The potency of the filtrate (toxin) was demonstrated by dermonecrotic and lethal tests on rabbits, and by hemolysin tests. It should be emphasized that rabbit erythrocytes must be used in all tests for staphylococcal hemolysin and antihemolysin, as the red blood cells of other animal species have been shown to give inconsistent results[9]—an observation which we have confirmed.

TABLE I.
Staphylococcal Antihemolysin Titers.

| | Total No. of Sera | 0.5 Internat. Unit or less | 1 Internat. Unit | 2 Internat. Units | Over 2 Internat. Units |
|---|---|---|---|---|---|
| Normal sera | 14 | 12 | 2 | 0 | 0 |
| Wassermann sera | 46 | 42 | 4 | 0 | 0 |
| TB osteomyelitis | 4 | 4 | 0 | 0 | 0 |
| Streptococcal infections | 7 | 4 | 1 | 2 | 0 |
| Staphylococcal infections | 25 | 15 | 3 | 3 | 4 |
| Osteomyelitis | 80 | 39 | 15 | 10 | 16 |

Table I gives the results of titrations of sera from the various groups studied. The "normal" sera were obtained from persons with no history of immediately preceding staphylococcal infection. The Wassermann sera were taken at random from sera received in the laboratory for the routine Wassermann test. The diagnosis of tuberculous or streptococcal infection was confirmed by the demonstration of the responsible bacteria in smear or culture. Demonstration of the etiologic agent in the staphylococcal infections and in the cases of osteomyelitis was obtained by the isolation of *Staphylococcus aureus* in every instance. The infections, other than osteomyelitis, included boils, furunculosis, cellulitis, lymphangitis, pyemia or septicemia, and infected wounds.

It will be noted that the titer of the great majority of sera from normal persons, or from non-staphylococcal infections, falls within the low range of 1 International Unit or less—well within the range reported as normal by Dolman and by Murray. Only 2 of 71 sera in this group had a titer over 1 International Unit; both sera came from cases of acute rheumatic fever.

In the cases of staphylococcal infections, other than osteomyelitis,

[9] Dolman, C. E., *Canad. Pub. Health J.*, 1932, **23**, 125.

the titer ranged from 0.5 International Unit or less to 22.7 units, with the majority of sera giving a titer of not over 1 International Unit. The titers of 3 sera from cases of boils or furunculosis were 0.5 International Unit. Of 4 sera with titers over 2 International Units, 2 were obtained from cases of septicemia, (4 units) and 2 from wound infections (3 and 22.7 units).

Of the 80 sera from cases of osteomyelitis, 54 gave a titer of 1 International Unit or less, and 25 gave a titer of 2 or more units. One-fifth of the sera had a titer of 3 or more units. Of this latter group, the 2 highest titers obtained were 12.2 and 17.7 units, while the majority ranged around 3 or 4 units. The average titer of this group was 5.1 International Units. It should be noted that all of the cases of primary acute osteomyelitis and most of the acute exacerbations of chronic osteomyelitis are included in this latter group, while of the group with 1 unit or less, all but 7 or 8 were chronic, and the majority had had no recurrence for a considerable period.

It is our opinion, from the results described above, that the "normal" staphylococcal antihemolysin value ranges up to about 1 International Unit. In the case of non-osteomyelitic staphylococcal infections, titers of sera from the majority of cases fall within the normal range, but a few will give titers definitely above normal. These findings appear to confirm, in general, the reports of Dolman, and of Murray.

In contrast to the uniformly high titers obtained in osteomyelitis by Dolman and by Murray, we found that the titer of the sera of about 2/3 of the patients fell within the normal range of 1 International Unit or less, and that the sera of only 1/5 of the patients possessed titers definitely above normal (3 units or more). In view of this, it would appear that the determination of staphylococcal antihemolysin can be of little diagnostic value in bone infections, as has been claimed by Murray.

The fact that low antihemolysin titers are so frequently found in staphylococcal infections does not necessarily indicate inability of the patient to produce antibody. This is shown in a series of about 35 patients with osteomyelitis who were treated with staphylococcal toxoid. In every instance the patient responded to the antigenic stimulus of the toxoid by the development of sera with antihemolysin titers ranging from 3 to 30 times the original titer. The details of this work are to be published later.

*Summary.* Normal sera possess a staphylococcal antihemolysin titer of about 1 International Unit or less. The titers of sera from our group of staphylococcal infections, other than osteomyelitis,

vary from 0.5 International Unit or less to 22.7 units, with the great majority falling into the normal range. Sera from 3 cases of superficial infections had titers of 0.5 unit.

In contrast to other reported results, about two-thirds of the sera from cases of osteomyelitis had a titer of 1 International Unit or less. Only one-fifth of the sera had definitely high titers, and these, in our series, averaged 5.1 units; the highest was 17.7 units. From our results, it seems to be necessary to modify the conception that a high antihemolysin titer is characteristic of osteomyelitis.

8381 P

## Further Studies on Transmissible Myelosis of Mice.*

W. A. BARNES AND J. FURTH.

*From the Department of Pathology, Cornell University Medical College, New York.*

It is now well established that lymphatic leukemia of mice is transmissible and neoplastic. Transmission of myeloid leukemia has only recently been accomplished.[1, 2, 3] The transmissible strain previously described from this laboratory[1] originated in a non-irradiated mouse. The basophile myelocytes characteristic of this strain produce myeloid leukemia in some animals, and multiple myeloma in others. Inoculations were successful in 39% of non-irradiated, closely related mice (Stock A) injected with these myeloid cells, and in only 15% of non-irradiated unrelated mice (Stocks R and S). The number of successful transfers was greatly increased by exposing the animals to X-rays prior to inoculation.

The incidence of spontaneous myelosis was approximately 10 times greater among irradiated mice than among their unirradiated siblings.[1] The present communication deals with one of the 2 successful attempts to transmit myeloid leukemia that occurred in uninoculated X-rayed mice. The first strain of transmissible myelosis that was derived from an X-rayed mouse will be referred to as strain Rfb 117. The mouse from which it originated (No. Rfb

* This investigation has been supported by a grant from the Lady Tata Memorial Trust and by a Fund for the Study of Leukemia.

1 Furth, J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 923; *J. Exp. Med.*, 1935, **61**, 423.

2 Parsons, D., *J. Path. and Bact.*, 1935, **40**, 45.

3 Kaalung-Jørgensen, O., *Zeitsch. f. Krebsf.*, 1935, **42**, 393.

117) was irradiated at the age of 80 days with a single massive dose of X-rays (400 r). When 229 days old, myeloid leukemia was evident from the blood smear. The spleen was greatly enlarged, but the superficial lymph nodes were not enlarged. The mouse was killed *in extremis* 263 days after birth, at which time the leukocytes numbered 66,000 per cubic mm. with 32% basophile myelocytes. The results of inoculations with a myeloid cell suspension from the spleen of this mouse and of the successive subpassages are summarized in Table I. The technic of inoculation has been described elsewhere.[1] Approximately two-thirds of the injections were intravenous, one-third subcutaneous.

TABLE I.
Result of Inoculations.

| Family of mice | Irradiation before inoculation | No. of experiments | No. of mice injected | Successful injections No. | Successful injections % |
|---|---|---|---|---|---|
| Rf | Not irradiated | 9 | 21 | 20 | 95 |
| Rg | Not irradiated | 7 | 36 | 13 | 36 |
| Rf-Rg hybrid | Not irradiated | 3 | 18 | 18 | 100 |
| Rf-Rg hybrid | Irradiated | 2 | 7 | 6 | 85 |
| A | Not irradiated | 7 | 45 | 3 | 7 |
| A | Irradiated | 8 | 81 | 8 | 10 |

Almost every member of the inbred family, Rf, is susceptible to this strain (Table I); inoculations are less often successful in the distantly related Rg stock and still less in the unrelated mice of Stock A, whereas the strain of myelosis previously described was best transmitted to mice of stock A. Irradiation failed to increase noticeably the susceptibility of the unrelated mice of stock A to strain Rfb 117.

Attempts at transmission with material free from living myelocytes have been unsuccessful in 30 mice. In these experiments the cells were destroyed by exposure to —30°C. during 30 minutes. Tissues exposed to this temperature when incubated *in vitro* in tissue cultures gave no evidence of the presence of live cells, but live cells were recovered from tissues frozen to —8°C. during 30 minutes.

Table II is a summary of some significant characteristics of transmissible strains of myelosis Ar 117 and Rfb 117, including the morphological appearance of the malignant cells, gross anatomical changes, percentage of successful inoculations among related and unrelated mice, and the effect of X-rays in increasing susceptibility to transmissible myelosis. These two strains exhibit such conspicuous differences that they can be distinguished by examination of blood smears alone.

TABLE II.
Comparison of Two Strains of Myelosis.

| | Strain Ar 117 | Strain Rfb 117 |
|---|---|---|
| *Percentage of successful inoculations* | | |
| Stock A, not irradiated | 33 | 7 |
| Stock A, irradiated | 88 | 10 |
| Stock Rg, not irradiated | 33 | 36 |
| Stock Rf, " " | 0* | 95 |
| *Malignant cells* | | |
| Average size | 16 $\mu$ | 14.7 $\mu$ |
| Granules | Coarse | Medium |
| | Dark purple-blue | Purple-red |
| | Oxydase negative | Oxydase positive |
| *Anatomical changes* | | |
| Enlargement of liver and spleen | Moderate | Great |
| Enlargement of lymph nodes | " | Slight |
| Color of the myeloid growth | Gray-white | Gray, often greenish |
| Occurrence of multiple tumors | Common (attached to bones and in viscera) | Rare (only in viscera) |

*This group is small (8 mice) but the material produced myelosis in 7 of the 8 irradiated mice injected.

In tissue cultures the malignant myeloid cells of strain Rfb 117 exhibited active amoeboid motion, multiplied by mitotic division, and did not mature into polymorphonuclear leukocytes as would be expected from the studies of previous workers.[4]

*Summary.* A new transmissible strain of myelosis that originated in a mouse irradiated by X-rays is described. This strain possesses characteristics which differ from those of the strain previously observed. The immature myeloid cells peculiar to this strain reproduce themselves by mitotic division *in vitro* and *in vivo* and like malignant cells, fail to mature.

8382 P

## Measurement of X-Ray Absorption Coefficients in Tooth Sections.*

FRANKLIN HOLLANDER.

*From the School of Dental and Oral Surgery, Columbia University.*

The following is a preliminary report of a new method of determining linear X-ray absorption coefficients in plano-parallel ground sections of teeth. This coefficient is a precise measure of radio-

[4] Timofejewsky, A. D., and Benewolenskaja, S. W., *Arch. f. Exp. Zellf.*, 1929, **8**, 1.

* This work was done with the aid of a grant from the Commonwealth Fund of New York.

pacity, which, in radiographs of such sections, is usually interpreted as measuring degree of calcification. Variations in calcification of teeth may arise in several ways: (1) With increasing age there may occur a progressive diffuse deposition of calcium salts within the already calcified matrix. (2) Secondary deposition of calcium salts may occur locally in response to some abnormal condition. (3) Certain pathological conditions may cause local rarifactions. (4) Structures in the organic matrix may cause relative variations in calcification merely by spatial displacement of inorganic material.

Such variations in distribution of inorganic material correspond to differences in compactness or density of calcification; *i. e.,* in weight of calcium salt per unit volume. However, the qualitative nature of radiopacity as ordinarily observed in a photographic film limits its value as a criterion of density of calcification for experimental purposes. To offset this limitation, Warren *et al.*[1] have measured the radiopacity by means of a Capstaff-Purdy densitometer for determining density of silver grains in photographic films. The precision of their method is stated to be of the order of 5% for sections 1 mm. thick and areas 0.5 mm. in diameter. Actually, it is even poorer than this since they used a mean thickness value throughout the section but did not include its precision measure in that of the entire determination.

Our experience with grenz-ray radiography of ground sections of teeth suggested that differences of considerably less than 5% may be significant in studies of normal calcification. In order to measure such differences, a method has been developed for the measurement of radiopacity which is good to 0.5% or better. This method employs soft X-rays and is based on the principle of the double ionization chamber described by Becker[2] for measuring minute changes in X-ray intensity. The apparatus was changed completely, however, in order to adapt it to the special requirements of a routine dental investigation. It consists of 2 symmetrical ionization chambers with a common collecting plate and individual charged plates at equal but opposite potential. Since the charge on the collecting plate represents the difference between the electrical effects in the 2 chambers, a string electrometer is used as a null instrument. The chambers are so placed over the grenz-ray tube that the radiation strikes their apertures symmetrically. A specially constructed me-

[1] Warren, S. L., Bishop, F. W., Hodge, H. C., and Van Huysen, G., *Am. J. Roentgenol. and Radium Therapy,* 1934, **31**, 663.

[2] Becker, J. A., *Physical Rev.,* 1922, **20**, 134.

chanical stage, provided with coordinate scales in the usual way, supports the specimen directly under one aperture; under the other, which has the same diameter, there are placed standards of measurement made of aluminum foil. Thus the size of specimen on which measurements are made is determined by the aperture. A micrometric arrangement for varying the length of air column permits of interpolation. Before starting measurements, the mechanical stage is placed on a microscope and areas, designated by their coordinate scale readings, are chosen for study. These circular areas are recorded on a photomicrograph of the specimen. To determine thickness at each position, provision is made for cutting out each disc and measuring it with a micrometer caliper.

The resulting apparatus is essentially a comparator whereby a minute area of ground section can be compared with a series of calibrated standards until 2 are found which most nearly match the unknown. Interpolation between these 2 values is effected by means of the air column scales. Measurements of the absorption coefficients are relative, being expressed as number of micra of aluminum which possess the same absorptive capacity. The precision of the method is indicated by the following: In a series of duplicate determinations on 60 different areas, the S.D. was $\pm 0.2\%$ or less in all but 4 instances. Also, for a micrometer caliper reading of $300\mu$, the S.D. was almost never greater than $\pm 0.3\%$. Consequently, the precision measure of the entire determination is around $\pm 0.5\%$ for *circa* 0.25 mm.$^3$ (0.7 mg.) of tooth substance. Measurements on non-carious human teeth have already shown small but characteristic differences within individual sections, as well as striking similarities among different teeth. For an experimental study involving animals with smaller teeth, it is necessary only that the aperture size be reduced to 0.5 mm.

8383 C

## Chemical Nature of Catalase.

HENRY TAUBER AND ISRAEL S. KLEINER.

*From the Department of Physiology and Biochemistry, New York Homeopathic Medical College and Flower Hospital, New York.*

The chemical nature of catalase has been studied by a number of authors. The experiments of Zeile[1] and of Stern[2] indicate that the catalase molecule is probably a chromoprotein in which the hemin group is related to that of the natural blood pigments. Recently Agner[3] reported that if horse liver catalase is dialyzed against N/10 or N/100 HCl the enzyme splits into 2 inactive components, one of a low molecular weight which dialyses through the cellophane membrane and which is possibly hemin and another component which remains within the bag and which is a protein. If the 2 neutralized components are mixed, an active preparation is again obtained, according to Agner.

Having the intention of making certain studies on the basis of Agner's report, we first attempted to repeat his experiment. The technique is simple but we were not able to confirm his results. The only difference between his and our technique is in the species of animal from which the liver was derived. Horse liver, which Agner used, is practically unobtainable in our locality. We have, however, tried beef, rabbit, and rat liver, respectively. We followed the experiments of Agner in every detail, dialyzing the purified catalase against N/10 or N/100 HCl, for 10 to 48 hours. No splitting (inactivation) of the catalase could be obtained. With stronger HCl irreversible inactivation of the catalase took place, due to a marked decrease in pH inside the dialyzing bag.

Catalase comprises at least a hemin group and a protein group. Waenting[4] reported that catalase solutions could be digested by trypsin. This we can confirm and we have carried out the digestion in the following manner. To a beef liver catalase solution of pH 6.4 an equal volume of a 0.3% solution of trypsin (Fairchild Bros. and Foster) of the same pH is added. Within 3 hours at 35° the catalase is completely digested (inactivated). The catalase solution used was of such strength that it decomposed 50% of 10 cc. of a

[1] Zeile, K., *Erg. Enzymforschung*, 1934, **3**, 265.

[2] Stern, K. G., *Nature*, 1935, **136**, 302.

[3] Agner, K., *Z. Physiol. Chem.*, 1935, **235**, II.

[4] Waenting, P., *Fermentforschung*, 1916, **1**, 165.

0.02 N $H_2O_2$ solution per cc. at 0° in 10 minutes in the presence of 2 cc. 0.05 M phosphate-borate buffer of pH 6.4. Enzyme action was discontinued by the addition of 2 cc. of 20% $H_2SO_4$ and the undecomposed $H_2O_2$ titrated with N/10 $KMnO_4$.

To one volume of completely digested beef catalase, which had been boiled to destroy the protease, and containing the unchanged hemin group was added one volume of catalase which had been inactivated by bubbling $H_2S$ through and the excess of $H_2S$ removed with $N_2$. To another sample of digested catalase solution was added some catalase which had been treated with the minimum inhibitive amount of KCN solution. The mixed samples (containing the digested-boiled and the inactivated enzyme) were incubated for 2 hours. No reactivation of the catalase took place in either case.

We have now attempted to determine whether the protein shown to be present can be replaced by another. To some of the digested and boiled catalase containing the hemin component normal human plasma, egg albumin and milk respectively was added in order to replace the "carrier" of the enzyme. In no case could even a partial catalytic effect be observed.

It is interesting to note that about 30 years ago Battelli and Stern[5] found that catalase activity is influenced by a number of substances such as (a) *anticatalase* which is checked by (b) a thermolabile *philocatalase*. The philocatalase is activated by (c) a special thermostable *activator*. Philocatalase has the ability to activate inactivated catalase. This system of activators and inhibitors has been overlooked by practically all workers and it is quite possible that Agner's results may have been affected by these substances.

Our experiments do not exclude the possibility that the catalase molecule is a hemoglobin compound. It is undoubtedly of protein nature.

[5] Reviewed by Stern, L., *Biochem. Z.*, 1927, **182**, 139.

8384 C

# A Comparison of Resistance of Bacteria and Embryonic Tissue to Germicidal Substances. VII. Potassium Mercuric Iodide.

A. J. SALLE AND A. S. LAZARUS.

*From the Department of Bacteriology, University of California, Berkeley, and the George Williams Hooper Foundation for Medical Research, San Francisco.*

Mercuric iodide is relatively insoluble in water but is easily soluble in an aqùeous solution of potassium iodide. The complex salt potassium mercuric iodide, having the formula $K_2HgI_4$, is formed by the interaction of one molecule of mercuric iodide ($HgI_2$) with 2 molecules of potassium iodide (KI). The preparation contains about 25.5% of mercury.

Aqueous solutions of the salt have been extensively employed as disinfectants because they do not possess the corrosive action on instruments and tissues that characterizes aqueous solutions of mercuric chloride. Another advantage is that they do not precipitate albumin.

Potassium mercuric iodide is about one-half as toxic as mercuric chloride when administered to animals. In proportion to the mercury content, however, the 2 salts possess about the same toxicity.

In previous papers of this series[1-6] comparisons were made of the resistance of *Staphylococcus aureus* and embryonic chick heart tissue to phenol, Merthiolate, Metaphen, Mercurochrome, Hexylresorcinol, iodine, and iodine trichloride. Toxicity indices were determined by dividing the highest dilution of the germicide that killed the tissue by the highest dilution of the chemical showing no growth of the test organism. Theoretically the smaller the toxicity index the more nearly perfect the chemotherapeutic agent.

The methods employed were the same as those given in the first paper of this series. Wide jumps in the dilutions were first prepared to determine approximately the least concentration of the germicide required to destroy the bacteria in 10 minutes but not in 5 minutes. Usually one such preliminary series was sufficient. Occasionally a second series covering higher dilutions was necessary. Having determined the approximate amount of the germicide required, a series

[1-5] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 665, 937, 1057, 1119, 1481.

[6] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 8.

of dilutions covering a narrow range were then prepared to determine more accurately the least concentration necessary to kill the bacteria in the specified period of time. In every case the results were checked a second time. If the second series failed to check the first the tests were repeated until checks were obtained. The same procedure was followed to determine the least concentration of the germicide required to kill the living embryonic tissue, except that a period of 48 hours was used instead of 10 minutes.

A *Staphylococcus aureus* phenol coefficient was first determined for potassium mercuric iodide by the method of Reddish.[7] Phenol killed *Staphylococcus aureus* in a dilution of 1-65 in 10 minutes but not in 5 minutes. The highest dilution of potassium mercuric iodide required to kill the test organism under the same conditions was found to be 1-900. Therefore, the *Staphylococcus aureus* phenol coefficient was 13.8.

Lambert[8, 9] found that *Staphylococcus aureus* was killed by a 1-5,000 dilution of the germicide after one hour. Watson[10] stated that a 1-1,000 dilution killed *Staphylococcus aureus* in one hour; a 1-500 dilution killed *B. coli* in 10 minutes; and a 1-1,000 dilution required 24 hours to kill *B. subtilis*. The same author found that a 1-1,000 alcoholic solution of potassium mercuric iodide had more than 10 times the germicidal efficiency of a 1-100 solution of iodine in alcohol. Macfarlan[11, 12] also reported favorable results with potassium mercuric iodide when tested against several organisms. He concluded that potassium mercuric iodide is a powerful germicide exhibiting marked germicidal efficiency in high dilutions. Also, its potency is reduced to a relatively slight degree by the presence of organic matter. On the other hand, Candy and Bulloch,[13] in their work on the sterilization of catgut found that mercuric iodide, whether dissolved in a solution of potassium iodide or in methyl or ethyl alcohol, cannot be regarded as a germicide of any marked power.

Cultures were prepared from chick heart tissue obtained from

[7] Reddish, G. F., The Newer Knowledge of Bacteriology and Immunology, E. O. Jordan and I. S. Falk, University of Chicago Press, 1928.

[8] Lambert, R. A., *J. Exp. Med.*, 1916, **24**, 682.

[9] Lambert, R. A., *J. Am. Med. Assn.*, 1916, **67**, 1300.

[10] Watson, C. H., *Surg. Gynec. and Obstet.*, 1916, **22**, 114.

[11] Macfarlan, D., *J. Am. Med. Assn.*, 1914, **62**, 17.

[12] Macfarlan, D., *Am. J. Med. Sci.*, 1920, **159**, 586.

[13] Candy, H., and Bulloch, W., *Brit. J. Exp. Path.*, 1928, **9**, 179.

9-day-old embryos. The fragments of tissue were embedded in guinea pig plasma in Carrel flasks. The various dilutions of phenol and potassium mercuric iodide were made in dilute chick embryonic fluid. The plasma, after coagulation, was washed with Tyrode solution to remove the uncoagulable constituents, after which were added the various dilutions of germicide in embryonic fluid. Final observations were made at the end of 48 hours.

The results are summarized in Table I.

TABLE I.
Toxicity of Phenol and Potassium Mercuric Iodide to Chick Heart Tissue and Bacteria.

| Germicide | Highest dilution showing no tissue growth = A | Highest dilution showing no growth of *Staph. aureus* = B | Toxicity Index = A/B | *Staph. aureus* phenol coefficient |
|---|---|---|---|---|
| Phenol | 1-840 | 1-65 | 12.9 | |
| Potassium mercuric iodide | 1-12,000 | 1-900 | 13.3 | 13.8 |

Lambert[8, 9] found that human adult tissues were killed by one-half the concentration of germicide required to destroy *Staphylococcus aureus*.

It is concluded from the above results that potassium mercuric iodide is relatively very toxic and that it rated considerably lower than most of the germicides so far studied when tested by the tissue culture technique. Also, the figure for the phenol coefficient was next to the lowest. The germicides studied may now be placed in the following order on the basis of their toxicity indices: iodine .09; iodine trichloride 0.40; Hexylresorcinol 3.0; Metaphen 12.7; phenol 12.9; potassium mercuric iodide 13.3; Merthiolate 35.3; Mercurochrome 262.0.

8385 P

## Antidotal Action of Picrotoxin in Extreme Cases of Experimental Barbiturate Poisoning.

CHARLES R. LINEGAR, JAMES M. DILLE AND THEODORE KOPPANYI.

*From the Department of Pharmacology and Materia Medica, Georgetown University School of Medicine, Washington, D. C.*

About 50 years ago, central stimulants, particularly picrotoxin and coriamyrtin, were used to oppose the depression caused by aliphatic narcotics. Recently, Tatum and his coworkers[1, 2] tested the antidotal effect of a number of central stimulants with the exception of coriamyrtin in experimental barbiturate poisoning. They produced the poisoning by oral or intraperitoneal administration of the barbiturate and found that picrotoxin was the most effective antidote in both short and long acting barbiturate depressions. Following the barbiturate, picrotoxin was given by diverse routes in doses of 8.8 to 12 mg. per kg. This drug increased the percentage recovery of the animals, *e. g.*, in 7 rabbits given picrotoxin following a dose of 350 mg. of sodium barbital per kg., 4 recovered and 3 died, whereas in 6 controls, 1 recovered and 5 died. Also the average recovery time of the surviving animals was shortened by picrotoxin. However, this dose given by these authors is not in excess of the average fatal dose to any appreciable extent. Gower and van de Erve[3] used a larger dose of sodium barbital and injected the drug intravenously. In one dog, they injected 600 mg. of this drug at 3 different times allowing 5 to 9 days to intervene after the complete recovery of the animal from the preceding dose. In these 3 experiments, the amount of picrotoxin which they used over a period of 4 to 8 hours following the sodium barbital injection varied from 6 to 8.8 mg. per kg. The dog recovered in each case within 32 hours. The dose of sodium barbital used by these workers was at least 50% in excess of the average fatal dose and about 3 times the minimum anesthetic dose.

The following experiments were carried out to determine the upper limit of the antidotal efficacy of picrotoxin in acute poisoning produced by a long acting barbiturate, sodium barbital, and by a short acting barbiturate, sodium pentobarbital. In each case, the barbiturate was administered intravenously in divided doses, the

[1] Maloney and Tatum, *J. Pharmacol.*, 1932, **44**, 337.

[2] Maloney, Fitch and Tatum, *J. Pharmacol.*, 1931, **41**, 465.

[3] Gower and van de Erve, *J. Pharmacol.*, 1933, **48**, 141.

first being the minimum anesthetic dose. Then picrotoxin was given in doses sufficient to produce in the animal a state of mild twitchings or convulsions before injecting a subsequent dose of the barbiturate. The time of injection of the total dose of these barbiturates varied from 15 to 52 minutes. After the last dose of the barbiturate, the animal was kept with picrotoxin in a state of hyperexcitability shown by continual twitching or mild convulsions upon gentle sensory stimulation for a period of 12 hours after pentobarbital and about 40 hours after barbital administration. The amount of picrotoxin necessary to maintain this responsiveness decreased as the experiment progressed so the original single doses of 4 mg. of picrotoxin per kg. were gradually reduced to 1 mg. and less per kg. In cases where the respiration or heart failed other measures in addition to picrotoxin such as artificial respiration, strychnine and epinephrine injections, were resorted to in an attempt to save the animal's life but were usually ineffective, or effective only for brief periods. The results of these experiments are summarized in Table I.

TABLE I.
Survival of Animals Poisoned by Massive Intravenous Doses of Barbiturates Following Picrotoxin Treatment.

| Animal | Barbiturate* Administered | Total dose injected mg./kg. | Time required for injection min. | Picrotoxin* Total dose injected mg./kg. | Fate of animal† |
|---|---|---|---|---|---|
| Dog | Sodium barbital | 1000 | 18 | 58 | Recov. in 48 hr. |
| ,, | ,, ,, | 1250 | 20 | 90 | ,, in 72 hr. |
| ,, | ,, ,, | 1250 | 37 | 101 | ,, in about 70 hr. |
| ,, | ,, ,, | 1500 | 15 | 60 | Died in 1 hr. 15 min. |
| ,, | ,, ,, | 1500 | 20 | 76 | ,, in 3 hr. 25 min. |
| ,, | ,, ,, | 1650 | 22 | 20 | ,, in 1 hr. 30 min. |
| Dog | Sodium pentobarbital | 100 | 35 | 18 | Recov. in 12 hr. |
| ,, | ,, ,, | 137½ | 41 | 76 | Died in 11 min. |
| ,, | ,, ,, | 150 | 38 | 76 | ,, in 7 min. |
| ,, | ,, ,, | 150 | 52 | 58 | ,, in 23 min. |
| ,, | ,, ,, | 160 | 44 | 42 | ,, in 22 min. |
| Rabbit | Sodium pentobarbital | 100 | 15 | 24 | Recov. in 5 hr. 30 min. |

*All doses administered intravenously.
†Time after last injection of barbiturate.

This table indicates that dogs receiving 1250 mg. of sodium barbital per kg., which is about 4 times the average fatal dose, will survive with the aid of picrotoxin and this dose of sodium barbital marks approximately the limit of the antidotal power of picrotoxin. With pentobarbital, it is doubtful whether the animal's life can be saved with doses much in excess of 100 mg. per kg. Furthermore,

doses of picrotoxin even as high as 100 mg. per kg., administered during a period of about 24 hours, can be tolerated by the animals if they are in a state of great depression. After recovery, the animals appeared normal in all respects. Thus, the extreme doses of either barbiturates or picrotoxin do not seem to have any deleterious effect upon these animals.

Blood sugar determinations by the Folin-Wu method in 2 animals receiving sodium barbital and picrotoxin showed in one animal no significant change and in the other a marked increase in the blood sugar. The control blood sugar of the first animal receiving 1,000 mg. sodium barbital per kg. was 114 mg. per 100 cc. and varied from 99 to 122 mg. during the first 17 hours after the last dose of the barbiturate, while the blood sugar of the third animal in Table I, in which the control blood sugar was 89 mg. and the dose of the barbiturate higher, rose to a peak of 177 mg. in 4 hours and gradually fell to 138 mg. in 10½ hours. No further determinations were made on either animal. The body temperature was taken at intervals on the latter animal only and was found to be maintained at a remarkably constant level in spite of the depressant action of a large dose of sodium barbital.

The urinary excretion of massive doses of sodium barbital, *i. e.*, 1,000 to 1,250 mg. per kg., is given in Fig. 1. This shows that the kidneys of dogs are capable of excreting large amounts of barbital (calculated as sodium barbital) presented to them, such as 3.857 to 7.397 gm. or 39.7 to 52.8% of the administered dose within the first

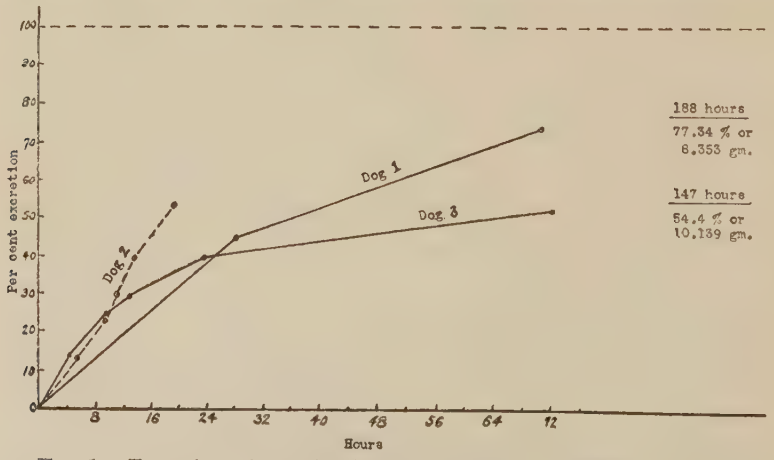


FIG. 1. *Excretion of massive intravenous doses of sodium barbital.* Dog 1, wt. 10.8 kg., received 1000 mg. sodium barbital per kg. or 10.8 gm. total; Dog 2, wt. 5.85 kg., received 1250 mg. sodium barbital per kg., or 7.313 gm. total, and Dog 3, wt. 14.9 kg., received 1250 mg. sodium barbital per kg., or 18.625 gm. total.

21 to 28 hours. This is comparable to the excretion of normal nitrogenous constituents as urea and creatinine. Since dogs 1 and 3 in Table I excreted 77.34 and 54.4% respectively of the administered dose up to the time of complete recovery of the animals and negative barbital findings in the blood and urine, it is to be assumed that fairly large amounts of this drug have been destroyed in the body. An indeterminable amount of the urine from dog 2 was lost after the first 21 hours after the administration of the barbiturate and hence the results are not tabulated after that time.

## 8386 P

### Carcinoma in the Cottontail Rabbit Following Spontaneous Virus Papilloma (Shope).

JEROME T. SYVERTON AND GEORGE P. BERRY.

*From the Department of Bacteriology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

The Shope rabbit papilloma is an epithelial new growth caused by a filtrable virus[1] and transmissible in series through cottontail (Genus, *Sylvilagus**)[1] and domestic rabbits (Genus, *Oryctolagus*).[2] Both in the gross and histologically it is similar to the virus papillomata of man, cattle and dogs.[1, 3–6] Further, it has been found to possess not only the immediate characters of a tumor,[7] but also the potentiality of progressive alteration until finally it may assume the characteristics of a squamous cell carcinoma with metastases.[8, 9] We have confirmed these findings.

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[1] Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

* Two species of cottontail rabbit, *Sylvilagus floridanus malluris* Thomas, and *Sylvilagus floridanus alacer* Bangs have been found suitable for serial transmission of the virus. We have confirmed the latter and can now report successful serial transmission through *Sylvilagus floridanus mearnsi* Allen, native to northwestern New York State.

We wish to thank Mr. George G. Goodwin of the Museum of Natural History, New York City, for the specific allocation of the cottontail rabbits used.

[2] Shope, R. E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 830.

[3] Ciuffo, G., *Gior. ital. mal. ven.*, 1907, **48**, 12.

[4] Magalhães, A., *Brazil-med.*, 1920, **34**, 430.

[5] Findlay, G. M., *Great Britain Med. Research Council*, 1930, **7**, 252.

[6] De Monbreun, W. A., and Goodpasture, E. W., *Am. J. Path.*, 1932, **8**, 43.

[7] Rous, Peyton, and Beard, J. W., *J. Exp. Med.*, 1934, **60**, 701.

[8] Rous, Peyton, and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 578.

[9] Rous, Peyton, and Beard, J. W., *J. Exp. Med.*, 1935, **62**, 523.

Carcinomatous changes have been reported previously only in experimentally inoculated domestic rabbits.[8, 9, 10] It would be highly significant were the host-parasite relationship so perfected in the natural host, the cottontail rabbit, that malignant changes never occurred. The significance of such a finding would apply irrespective of whether the virus *per se,* or whether secondary factors were responsible for the carcinoma. The purpose of this communication is to report that such is not the case, for we have observed carcinomatous degeneration of a naturally occurring papilloma in a wild cottontail rabbit.

Of 132 cottontail rabbits (*Sylvilagus floridanus alacer* Bangs) received from southern Kansas, 11 have had spontaneously one or more of the papillomatous growths. These 11 cottontails were kept for observation and for a source of virus. With a single exception, our findings have agreed with those previously described.[1, 7] The history of the exceptional case follows:

Cottontail rabbit S5-CR 363. Thin, young, adult male. Received March 30, 1935. On lower abdomen 4 pigmented warts were noted: A, 18x22x15 mm. in height, B, C and D, 4-6 mm. in diameter x6-8 mm. in height. Rapid gain in weight. Papillomata appeared unchanged for 85 days, when lesion A showed superficial ulceration with thickening of the underlying tissues. Six days later the rabbit died from fracture of the lumbar spine sustained accidentally the previous day.

Necropsy revealed a well developed and nourished adult cottontail rabbit. Externally the lesions were as noted. Sections were removed from each for histological study. In the left axilla an enlarged lymph node, measuring 7x7x12 mm. was found. It was irregular, but not indurated. No other lymphadenopathy or evidence of metastasis was encountered. Death had been caused by fracture of the lumbar spine with extensive hemorrhage.

Microscopic examination of the sections of lesion A and of the enlarged axillary node showed histological pictures entirely characteristic of squamous cell carcinoma.

A case is presented of carcinomatous change following a spontaneously occurring papilloma (Shope) in the cottontail rabbit, *Sylvilagus floridanus alacer* Bangs.

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[10] Beard, J. W., and Rous, Peyton, PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 191.

8387 P

# Susceptibility of Mouse Strains to Lung Tumor and Sarcoma Induced by 1:2:5:6-dibenzanthracene.

CLARA J. LYNCH.

*From the Laboratories of the Rockefeller Institute for Medical Research.*

It has been shown that strains of mice differ in their inherited susceptibility to tumors both spontaneous and tar-induced.[1] Burrows, Hieger and Kennaway[2] and others have demonstrated the effectiveness of 1:2:5:6-dibenzanthracene in inducing sarcoma. The present paper is a preliminary report on the tumors induced by subcutaneous injection of 1:2:5:6-dibenzanthracene into various strains of mice.

Ordinary lard was filtered at 37°C. and the crystalline dibenzanthracene was dissolved in the filtrate by reheating to 140°C. Each mouse received a total dose of 4 mg. of 1:2:5:6-dibenzanthracene in 1 cc. of lard administered in 3 subcutaneous injections of 0.25 cc., 0.25 cc., and 0.5 cc. at biweekly intervals.

Of the mice receiving treatment 2 groups were from our branch of the Bagg albino strain. The 3rd was from our "Yellow" strain which is only pen inbred and probably not very homogeneous genetically but interesting because it produces yellow and brown mice. A 4th was from No. 1194—our highly inbred agouti or wild type strain; a 5th from Strain 5—an imported albino stock from McGill University. The 5th contained males only. The 6th was from Strain 62 which is composed of pink-eyed dilute brown mice, highly

TABLE I.

Percentage of Tumor after Subcutaneous Injection of 1:2:5:6-dibenzanthracene in Mice from Various Sources.

| | Sarcoma | | | Lung tumor | | |
|---|---|---|---|---|---|---|
| Strain | No. mice | No. with tumor | % tumor | No. mice | No. with tumor | % tumor |
| Bagg (previous experiment) | 60 | 45 | 75.0 | 59 | 48 | 81.4 |
| Bagg (Group I) | 44 | 33 | 75.0 | 46 | 41 | 89.1 |
| Bagg (Group II) | 29 | 26 | 89.7 | 29 | 25 | 86.2 |
| Yellow | 32 | 29 | 90.6 | 30 | 10 | 33.3 |
| 1194 | 10 | 9 | 90.0 | 12 | 2 | 16.7 |
| 5 | 33 | 29 | 87.9 | 31 | 2 | 6.5 |
| 62 | 18 | 15 | 83.3 | 18 | 0 | 0.0 |

[1] Lynch, C. J., *J. Exp. Med.*, 1931, **54**, 747; Proc. Soc. Exp. Biol. and Med., 1933, **31**, 215.

[2] Burrows, H., Hieger, I., and Kennaway, E. L., *Am. J. Cancer*, 1932, **16**, 57.

inbred brother by sister. Another group of Bagg mice from a previous experiment may be legitimately included for discussion here.

The results are shown in Table I. The presence of lard makes it difficult to determine the time when malignancy supervenes in the injected tissue, and lung tumors are found only at autopsy. Therefore the percentages are based upon the populations surviving at the time the presence of the first tumor of each type was proven by microscopic sections. For the lung tumor, this date was the 66th day after the last injection; for the sarcoma, the 73rd day. No mice lived longer than 376 days after treatment, the majority less than 200 days. The table lists the subcutaneous sarcomas occurring at the site of injection and primary lung tumors of epithelial origin.

All groups show a high incidence of sarcoma. Though there is a distinct variation no strain can be clearly demonstrated to have a significantly different rate from any other.

The distribution of lung tumors is quite different, ranging from 0.0 to 89.1%. While the Bagg groups have about the same rate, each is significantly different from all of the 4 other stocks. The Yellow stock has an intermediate rate which is significantly different from all but Strain 1194. A detailed analysis shows that strain differences are not merely the result of variations in length of life after treatment. For example, by the 158th day when the mice of Strain 1194 were dead, with a lung tumor rate of 16.7%, the 71 mice that had died in the 3 Bagg groups showed 74.6%. Thirteen mice of Strain 62 lived well beyond this time but none developed a tumor.

If a comparison is made of the susceptibility to the 2 kinds of tumor exhibited by each stock it is found that the Bagg groups are highly susceptible to both. The greatest divergence, found in Group I, is not mathematically significant. But it is evident by inspection that the last 4 groups exhibit a marked difference in the reaction of subcutaneous connective tissue and pulmonary epithelium.

TABLE II.

Percentage of Spontaneous Tumors in Various Strains of Mice.

| Strain | Total No. of mice | No. with sarcoma | % sarcoma | No. with lung tumor | % lung tumor |
|---|---|---|---|---|---|
| Bagg (1931-34) | 207 | 0 | | 66 | 31.8 |
| 1194 (1931-34) | 327 | 1 | 0.31 | 8* | 2.4 |
| 62 | 199 | 0 | | 12* | 6.0 |
| 5 | 50 | 0 | | 2? | 4.0? |

* Includes questionable cases.

The data are based upon mice over 6 months old. Those for the Bagg strain and 1194 are from May, 1931, through December, 1934.

In attempting to explain these results reference may be made to the records of spontaneous tumor in 4 of these strains. (No data are available for the "Yellows".) The data (Table II) are based upon mice over 6 months old. Earlier records of the Bagg and 1194 strains have shown a small percentage of sarcoma (less than 2%). The later stock records given here are selected as being a closer control.

Only one spontaneous sarcoma occurred in these groups. No difference can be demonstrated between strains just as no difference was demonstrated between strains for induced sarcoma. However, as regards lung tumor the strains are dissimilar. The Bagg strain which here shows 31.8% gave 81.4 to 89.1% after dibenzanthracene. Evidently subcutaneous injection of dibenzanthracene induces lung tumor as well as sarcoma. The Bagg strain is also plainly more susceptible to spontaneous lung tumor than are the other 3 strains just as it was more susceptible to induced lung tumors than were the other strains.

These results confirm our earlier conclusions that strains differ in susceptibility to induced tumor and that susceptibility is organ-specific.

A number of other tumors occurred in these experiments but they will be treated more fully later. Mention might be made, however, that a mammary tumor occurred in a male in Strain 5.

8388 P

## Skin Reactions in Sarcoid.

R. H. WILLIAMS AND D. A. NICKERSON. (Introduced by R. N. Nye.)

*From the Mallory Institute of Pathology, Boston City Hospital.*

Based on the hypothesis that sarcoid, like lymphogranuloma inguinale, might be a virus disease and that, like the latter, an antigen could be prepared from infected material for use for diagnostic purposes, the following work was done.

A sarcoid lesion of the skin, 2 cm. in diameter, was removed from the first patient described below. A portion of the tissue was taken for microscopic study, while the remainder was ground with the aid of sterile sand. To this, normal saline was added in a volume equal to 6 times that of tissue. This preparation was then sterilized

by heating to 60°C. for 2 hours on 2 consecutive days. Aerobic and anaerobic cultures were negative at the end of 48 hours.

In each of the following cases, stained sections and animal inoculation of the tissue removed were negative for tuberculosis. The patients showed no evidence of tuberculous infection by clinical examination, cutaneous tests or X-ray examination.

Case No. 1. A 25-year-old female, who 4 years ago had her spleen removed. The spleen weighed 540 gm. Microscopically, it was studded with round, well defined, single or confluent, large, inflammatory lesions consisting of fibrous tissue, elliptical macrophages and a few lymphocytes. About the periphery were a few lymphocytes, macrophages and eosinophils. A few of the lesions contained multinucleated giant cells. The microscopic diagnosis was sarcoid.

Two months after splenectomy, a skin lesion appeared on the left forearm, and throughout the last 4 years additional lesions have appeared, which slowly, but progressively, enlarged.

Many firm, elevated, bluish-red, irregularly discoid lesions, 0.3-2.0 cm. in diameter, were scattered over the face, arms, and, to a lesser degree, the legs and buttocks. Physical examination revealed no other abnormalities except an enlarged liver, which extended to the umbilicus and right iliac crest.

Microscopic examination of the lesion removed by biopsy revealed a picture similar to that in the spleen.

The patient was injected with 0.05 cc. of the antigen intradermally. Within 24 hours, a firm red papule, 3 mm. in diameter, appeared. It increased slowly in size until the end of 36 hours and then slowly regressed. At the end of a week, there was still a small papule. Two days after the first, a second injection of 0.10 cc. resulted in a larger papule with a narrow zone of erythema. Three subsequent injections, given at 3-day intervals, produced similar reactions.

Within 3 weeks, it was noticed by several observers, each unaware that treatment had been given, that there was a definite decrease in size and redness of the skin lesions.

Case No. 2. This 13-year-old white girl had a splenectomy 4 years ago with a biopsy of the liver and a mesenteric lymph node. The spleen weighed over 500 gm. Histologically, the spleen, the liver and mesenteric node showed lesions similar to those in the preceding case, interpreted as sarcoid.

The patient was given 0.15 cc. of the antigen intradermally and, within 24 hours, a firm red papule had formed, surrounded by a

zone of erythema. At the end of 36 hours, the area of erythema was 1.5 cm. in diameter and the papule 7 mm. in diameter. The erythema lasted 48 hours, the papule 8 days.

Case No. 3. This was a young adult female who within a few months had a resection of 23 cm. of ileum and colon. There was marked thickening and induration of the intestinal wall in the region of the ileo-cecal valve. Microscopic examination showed lesions similar to those above, though not as numerous.

Four weeks after operation, 0.10 cc. of antigen was administered intradermally. Within 24 hours, a firm red papule developed; in 36 hours, it was 5 mm. in diameter and was still present after a week.

Case No. 4. This middle-aged white female had a resection of a loop of intestine 2 years ago following a clinical diagnosis of "regional ileitis." Microscopically, the lesions were similar to those in the preceding cases and were interpreted as sarcoid.

Intradermal injection of 0.10 cc. of antigen produced a firm, red, elevated papule, 6 mm. in diameter, at the end of 36 hours.

Four normal healthy adults were used as control cases. They were given 0.10 cc. of the antigen intradermally, and were completely negative at the end of 36 hours. Two of these individuals had positive tuberculin reactions, and the third had had active tuberculosis 2 years ago.

The above cases, while not sufficient to offer conclusive evidence, suggest that the hypothesis that sarcoid is a virus disease may be correct, and that an antigen may be prepared for diagnostic purposes. More extensive study is being carried out and will be reported later.

*Summary.* (1) Four cases with clinical and pathologic evidence of sarcoid gave skin reaction following the intradermal injection of an antigen made from a sarcoid lesion of the skin. Four normal individuals gave no such reaction. (2) These results suggest that sarcoid is a virus disease and that it is possible to prepare a diagnostic antigen.

8389 C

## Decreased Mammotropin in Pituitaries of Thyroidectomized (Maternalized) Male Rats.

MORVYTH MC QUEEN-WILLIAMS. (Introduced by Herbert M. Evans.)

*From the Institute of Experimental Biology, University of California.*

The attitude[1] of adult male rats may be so altered that they will display maternal interest in newborn rats. This can be accomplished by complete ablation of the thyroid, or by administration of bovine anterior hypophyseal implants.

The anterior pituitaries of maternalized male rats, thyroidectomized 3-4 months previously (at the end of first month of life), were assayed for lactogenic hormone by the squab local crop sac method. Implants were made subcutaneously into one-month-old pigeons, glandules from normal rats being inserted directly over the center of one side of the crop sac, as controls to the hypophyses from thyroparathyroidectomized rats, which were implanted at a similar site on the opposite side. Each bird was implanted once on each side. At the end of 96 hours, the pigeons were sacrificed and the crop sacs examined, graded and preserved, stretched over a cork, in 10% formol.

The anterior pituitary of one normal male rat is usually sufficient to induce a good response in the pigeon crop sac. In positive reactions, a circumscribed, thickened, opaque, well vascularized area can be seen just under the implant site, easily distinguished from the rest of the thin transparent membrane. The right and left sides can be studied independently, each bird serving thus as its own control, unless an excess of lactogenic hormone is administered, which would result in a change involving the whole of one side and spreading to the other. The greatest reaction in this series (Table I) was obtained by implanting 2 normal male hypophyses on one side. The opposite side, which received the pituitaries from 2 thyroidectomized rats, exhibited no noticeable response. In all cases, the soft, swollen, purplish hypophyses from the thyroidectomized male rats induced a negative or only a slight response, in spite of their greater weight.

The definitely subnormal store of lactogenic hormone in the pituitaries of thyroidectomized male rats might be explained by the conception that this hormone is being used up rapidly in these animals that manifest such unusual behavior.

[1] McQueen-Williams, M., *Science*, 1935, **82**, 67.

TABLE I.

Anterior Pituitaries of Thyroidectomized (Maternalized) Male Rats Tested for Mammotropin.

| Pigeon | Sex | Donors of Pituitaries (male rats 4-5 mos. old) No. | Body wt. (gm.) | Maternal behavior | Wt. of implanted tissue (mg.) | Crop Sac Reaction |
|---|---|---|---|---|---|---|
| | | | | Thyroidectomized. | | |
| 1 | ♂ | 1 | 216 | Yes (good) | 10.0 | Slight (+) |
| 4 | ♂ | 1 | 214 | " (very good) | 12.0 | " (+) |
| 5 | ♂ | 1 | 240 | " (fairly good) | 11.0 | No |
| 10 | ♂ | 1 | 212 | " " " | 15.0 | " |
| 12 | ♂ | 1 | 215 | " " " | 12.5 | Slight (+). Diffuse. |
| 25 | ♂ | 2 | 218 | " (fair) | 11.0 | " (+) " |
| | | | 217 | " " | 12.3 | |
| P | ♂ | 2 | 266 | " (fairly good) | 15.0 | No |
| | | | 222 | " (fair) | 15.0 | |
| LC | ♀ | 1 | 212 | " (very good) | 9.0 | No |
| 2 | ♀ | 1 | 190 | " (good) | 11.5 | " |
| 3 | ♀ | 1 | 214 | " (very good) | 15.0 | Slight |
| 6 | ♀ | 1 | 229 | " (fairly good) | 7.7 | " (+). Diffuse. |
| 11 | ♀ | 1 | 213 | " " " | 8.0 | " |
| | | | | Normal. | | |
| 1 | ♂ | 1 | 380 | No | 8.3 | Good (++) |
| 4 | ♂ | 1 | 321 | " | 7.9 | Very good (+++) |
| 5 | ♂ | 1 | 384 | " | 7.3 | Slight (?) |
| 10 | ♂ | 1 | 362 | " | 7.5 | No |
| 12 | ♂ | 1 | 326 | " | 6.5 | Good (++). Diffuse. |
| 25 | ♂ | 2 | 308 | " | 6.0 | " (++) |
| | | | 304 | " | 5.5 | |
| P | ♂ | 2 | 333 | " | 7.0 | Very good (++++)* |
| | | | 354 | " | 7.3 | |
| A | ♂ | 1 | 370 | " | 5.3 | Slight (+) |
| A | ♂ | 2 | 360 | " | 5.3 | Good (++) |
| | | | 330 | " | 5.3 | |
| LC | ♀ | 1 | 334 | " | 9.0 | No |
| 2 | ♀ | 1 | 380 | " | 7.7 | " |
| 3 | ♀ | 1 | 308 | " | 7.0 | Good (++) |
| 6 | ♀ | 1 | 450 | " | 8.2 | " (++). Diffuse. |
| 11 | ♀ | 1 | 369 | " | 7.8 | " (++) |

*Most of crop sac on one side responded.

The writer is indebted to Dr. W. R. Lyons of this laboratory for his kindness in explaining and permitting the use of this assay method devised by him (as yet unpublished).

*Summary.* The anterior pituitaries of normal adult male rats, when implanted directly over the center of the crop sacs of squabs, evoke a greater response in the crop sacs, hence store more mammotropin, than do the glandules from thyroparathyroidectomized (maternalized) males of the same age.

*Acknowledgment* is hereby made to Dr. Herbert McLean Evans for his helpful criticism.

8390 C

## Effect of Ultraviolet on Heart of Rana Pipiens and Alligator Mississippiensis.

S. A. GUTTMAN. (Introduced by H. S. Liddell.)

*From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.*

Hinrichs and Johnson[1] observed that "short exposures in the region of the sino-auricular node of both frog and turtle produced a noticeable increase in rate." Also, these investigators found that "it has been possible to stimulate the heart of a frog to regular beat, normal in sequence and in amplitude, by radiation at the sino-auricular node after the heart has been quiescent for an hour or more." They did not report any amplitude changes after radiation of the normal heart.

The effect of ultraviolet radiation on the exposed heart of the pithed frog, *Rana pipiens,* and decapitated alligator, *A. mississippiensis,* immersed in Ringer's solution which was kept at a fairly constant temperature of 12-14°C., was observed during irradiation by a Cooper-Hewitt Quartz Mercury Arc or a General Electric S.1 lamp at 50 cm. from the preparation. The former was the source employed unless otherwise stated.

Several series of experiments were performed and the following facts were noted:

1. Freshly prepared normal hearts were found to exhibit a slight increase in frequency and amplitude upon irradiation for short intervals (5-10 minutes) after which both indices rapidly fell below the normal and exhibited a delayed and slow recovery. A normal untreated preparation, under the conditions employed during the course of this investigation, exhibits about a 10% fall in amplitude after 3 to 4 hours while the change in frequency may be regarded as negligible.

Relatively long irradiation (16 minutes) produced a rise in frequency and amplitude and the subsequent rapid fall but, when a long irradiation (63 minutes) was given before the usual slow recovery set in, the frequency and amplitude kept falling. It was not until 2 hours after irradiation ceased that the indices showed signs of recovery and a return to normality.

When a fairly short intense irradiation (8 minutes) and 2 long,

[1] Hinrichs, M. A., and Johnson, P. O. C., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 971.

relatively weak ones (1 hour each) from a General Electric S.1 lamp were given it was observed that the short intense irradiation did not affect the preparation deleteriously and thus the responses to the weak, long irradiations exhibited the same effect as was noted to be the case when short, intense irradiations were employed.

2. Several hearts were immersed in cool (12-14°C.) Ringer's solution until they ceased beating. These were then irradiated and they started to beat after from one to 10 minutes of irradiation. On one heart this irradiation procedure was carried on for a period of 70 hours.

3. Records were obtained of hearts which exhibited an abnormal rhythm and it was noted that short dosages of ultraviolet were quite effective in inducing a normal rhythm and this did not revert to the original state after the cessation of radiation. Also, a slight increase in tonus appeared.

8391 P

## Normal Heart Weight/Body Weight (HW/BW) and Left to Right Ventricular (L/R) Ratios for Rabbits.*

GEORGE HERRMANN, GEORGE DECHERD, PETER ERHARD AND E. H. SCHWAB.

*From the Department of Medicine, University of Texas, Galveston.*

In order to have adequate controls for further experimental studies upon cardiac hypertrophy we have considered it necessary to accumulate data from a large series of normal rabbits and to treat this data by statistical methods. Many previous investigators have published average heart weight body weight ratios for small series of rabbits, but there are no figures for the ratio of the left to the right ventricular weights. Hasenfeld and Romberg[1] in a control series of 32 normal rabbits found an average of 2.38 gm. of heart muscle per kilo of body weight. Joseph[2] in a series of 38 male and 66 female rabbits found an average ratio of 2.67 in the males with a maximum of 3.42 and a minimum of 2.07, while for the females the average ratio was 2.70 with a maximum of 4.47 and a minimum

* Supported in part by Grant No. 349 from the Committee of Scientific Research of the American Medical Association.

1 Hasenfeld, A., and Romberg, E., *Arch. f. exp. Path. u. Pharm.*, 1897, **39**, 333.

2 Joseph, D. R., *J. Exp. Med.*, 1908, **10**, 521.

of 2.0 gm. of heart per kilo body weight. Wassermeyer and Rohrbach[3] quote Kuelb's average figure on a small series of normal domestic rabbits at 2.70 and Hesse's average figure of 2.70 on a similar series and their own figures on 10 rabbits with a maximum of 2.73 and a minimum of 2.03 and an average of 2.38 gm. of heart per kilo of body weight. Sekiguchi[4] used as control his average of 2.04 gm. of heart per kilo of body weight obtained from the study of 10 normal rabbits.

In a series of 168 normal rabbits of Chinchilla, New Zealand, Red, White and Dutch Black strains and mixtures of these, with body weights varying from 900 to 3,000 gm. we have found the arithmetical mean of the HW/BW to be 1.972 gm. heart per kilo body weight, with a standard deviation of 0.299 and a probable error of ±0.015 for the mean and ±0.011 for the standard deviation. We also dissected 101 of these hearts in the fresh and divided them by the authors'[5] midseptal method into auricles, left and right ventricular masses. The left to right ventricular ratios (L/R) mean was 1.792 with a standard deviation of 0.138 and probable errors of ±0.0092 for the mean and ±0.0065 for the S. D. The mean normal auricular weight body weight ratios were 0.270 with an S. D. of 0.054 and for the auricular weight heart weight ratios mean was found to be 137 ± 23.

8392 P

## Experimental Ablation of Posterior as Contrasted to Anterior Aortic Cusp on Cardiac Hypertrophy in the Rabbit.*

E. H. SCHWAB, GEORGE HERRMANN AND J. FRANK CONNALLY, JR.

*From the Department of Medicine, University of Texas, Galveston.*

During the progress of studies on the results of digitalization on experimental aortic insufficiency hypertrophy it was observed that the gross configuration of the hypertrophied heart appeared to differ. Some were globular in form whereas others were elongated or boot

[3] Wassermeyer, H., and Rohrbach, A., *Arch. f. exp. Path. u. Pharm.*, 1932, **166**, 375.

[4] Sekiguchi, R., *Ber. ges. Physiol.*, 1935, **87**, 1928.

[5] Herrmann, George, *Am. Heart J.*, 1925, **1**, 485.

* Supported by Grant No. 349 from the Committee on Scientific Research of the American Medical Association.

TABLE I.
Cardiac Hypertrophy as Influenced by Location of Aortic Defect.

| No. cases | HW/BW | L/R | A/BW | A/HW | Duration, wk. | Extent of lesion |
|---|---|---|---|---|---|---|
| | | | Anterior Cusp. | | | |
| 7 | 2.21 | 2.10 | .305 | 137. | 16 | Small |
| 8 | 2.21 | 1.86 | .286 | 129.5 | 8 | " |
| 11 | 3.40 | 1.86 | .535 | 164.5 | 12 | Complete |
| 21 | 3.70 | 2.30 | .430 | 111. | 15 | " |
| 22 | 2.07 | 1.88 | .292 | 141. | 16 | Small |
| 35 | 3.45 | 2.01 | .467 | 135. | 15 | Complete |
| 38 | 3.38 | 2.15 | .423 | 125. | 15 | " |
| 51 | 3.71 | 2.16 | .577 | 156. | 10 | " |
| 53 | 2.76 | 1.95 | .256 | 93. | 14 | Moderate |
| 62 | 2.29 | 1.73 | .317 | 138. | 9 | Small |
| 63 | 3.20 | 1.87 | .360 | 111. | 11 | Moderate |
| 64 | 2.55 | 1.76 | .290 | 113. | 10 | " |
| 66 | 3.05 | 1.83 | .295 | 97. | 14 | " |
| 67 | 4.68 | 1.94 | .840 | 180. | 14 | Complete |
| 74 | 2.72 | 1.80 | .359 | 130. | 13 | Moderate |
| 76 | 2.18 | 2.00 | .273 | 125. | 13 | Small |
| Aver. | 2.98 | 1.94 | .394 | 130. | | |
| | | | Posterior Cusp. | | | |
| 12 | 3.15 | 1.76 | .520 | 165. | 8 | Complete |
| 15 | 3.26 | 1.93 | .474 | 145. | 16 | " |
| 19 | 3.34 | 1.88 | .380 | 113. | 15 | " |
| 20 | 3.80 | 2.01 | .369 | 97. | 15 | " |
| 23 | 3.57 | 1.71 | .500 | 140. | 15 | " |
| 27 | 4.35 | 1.32 | .835 | 192. | 3 | " |
| 49 | 2.42 | 1.95 | .246 | 101. | 14 | Moderate |
| 72 | 4.28 | 1.84 | .530 | 124. | 13 | Complete |
| 77 | 3.53 | 1.29 | .800 | 227. | 2 | " |
| 78 | 2.85 | 1.84 | .363 | 127. | 13 | " |
| 79 | 4.10 | 1.69 | .815 | 198. | 12 | " |
| 117 | 3.07 | 1.53 | .618 | 201. | 2 | " |
| Aver. | 3.48 | 1.72 | .537 | 157. | | |
| | | | Combined Lesion. | | | |
| 34 | 4.37 | 1.94 | .578 | 170. | 15 | Ant. complete + post. moderate |
| 39 | 5.58 | 1.88 | .727 | 130. | 11 | Post. complete + ant. moderate |
| 42 | 3.14 | 1.72 | .311 | 99. | 15 | Post. complete + ant. small |
| 102 | 4.15 | 1.39 | .810 | 195. | 5 | Post. complete + ant. small |

shaped. The hearts were dissected by removal of the auricles and division of the ventricular mass by the midseptal method. It was found that the globular heart was associated with ablation of the posterior leaflet and the right ventricular wall appeared to be considerably thickened along with the increased mass of the left heart. The *coeur en sabot* type, on the other hand, was found occurring usually in those that had destruction of the left anterior cusp. In the latter group the thickness of the right ventricular wall did not appear to be conspicuously increased in thickness, while the left

ventricular wall was strikingly so. In a few hearts with combined lesions the configuration depended upon which leaflet damage was the more extensive. The obvious visual impression was substantiated by the weights of the ventricular masses and the ratios of these to one another as shown in Table I.

The hypertrophy of the auricles was observed to be greater in the hearts with posterior incompetency and the auricular body weight and the auricular heart weight ratios seem to confirm this. The heart weight body weight ratios show that the amount of total hypertrophy is almost uniformly greater in the hearts with posterior lesions. Difference in the intracardiac dynamics of the circulation with the two types of aortic regurgitation is the probable factor. A relative mitral stenosis (Austin Flint phenomenon) is a possible explanation of concomitant auricular and right ventricular hypertrophy in the presence of the regurgitation through the posterior sector.

# Minnesota Section

*University of Minnesota, November 20, 1935.*

8393 P

## Separation of the "Colloid" from Living Thyroid Gland by Means of Centrifugal Force.

J. F. MC CLENDON.

*From Fysikalisk-Kemiska Institutionen, Upsala Universitet.*

Following the lead of E. P. Lyon[1] and others, in some previous work I have shown that it is possible by means of a centrifugal force of about 3,000 times gravity to cause separation of heterogeneously dispersed materials inside living cells into distinct layers, depending on their density, without killing the cells, even though they were kept in the centrifuge for a week. In later centrifuge experiments[2, 3] I separated crystals appearing homogeneous in the polarizing microscope and isolated them for chemical analysis. When these crystals were treated with alcohol they were decomposed into a phospholipin and a vitellin. Cavett, Rice and McClendon[4] reported that thyroglobulin treated with acetone and ether (as a substitute for alcohol, which denatured it) lost weight (increased in iodine percentage), which I interpreted as due to splitting off of some lipin. To test this hypothesis some method of getting thyroglobulin from thyroid without contamination with cell proteins was needed.

Thyroglobulin, the protein of the thyroid gland, is present in the sol or gel known as "colloid", which is contained in the thyroid follicles. The follicles containing "colloid" are of a spheroidal shape formed by a single layer of flattened or cuboidal cells. Since spaces between these cells have been reported by cytologists it occurred to

[1] Lyon, E. P., *J. Exp. Zool.*, 1909, **6**, 269.

[2] McClendon, J. F., *Arch. Entwicklungsmech.*, 1909, **27**, 247.

[3] McClendon, J. F., *Am. J. Physiol.*, 1908, **23**, 460; 1909, **25**, 195.

[4] Cavett, Rice and McClendon, *J. Biol. Chem.*, 1935, **110**, 673.

me that by the use of a sufficiently high centrifugal field, the "colloid" might be removed from the follicle.

When a portion of rabbit thyroid tissue was subjected to the centrifugal force of 160,000 to 200,000 times gravity in the ultracentrifuge at Fysikalisk-Kemiska Institutionen, Upsala, a fluid separated, forming a layer above the tissue. If the centrifugal force of 100,000 times gravity is prolonged, one can observe the refraction of the boundary of sedimenting protein. The sedimentation velocity of this protein was obtained through an ultracentrifugal study of a diluted portion of the fluid and was found to correspond to the value previously obtained at this laboratory for thyroglobulin.[5, 6]

The separated fluid contained a small amount of hemoglobin which was evident from its color. When a portion of a rabbit gland perfused with saline was subjected to the ultracentrifugal field a fluid was obtained which was apparently free from hemoglobin. Microscopic sections showed that many of the follicles had flattened during the procedure. Thyroglobulin was also obtained from hog and human thyroid tissue by centrifugal force.

If the molecules of thyroglobulin passed through the cells on their way out of the follicle they would pass in a centrifugal direction, whereas we found the thyroglobulin passed out in a centripetal direction. Therefore it was not the thyroglobulin that moved but the cells that were acted on by the centrifugal force and precipitated as units. If they were cemented together in continuous sacks they would be buoyed up by the colloid. Therefore there are spaces between the cells.

I wish to express my indebtedness to Professor The Svedberg, Dr. K. Pedersen, Dr. H. Lundgren and others of Fysikalisk-Kemiska Institutionen, and to Professor Agduhr of Histologiska Institutionen for their assistance in this investigation, which is being continued under a grant from the Rockefeller Foundation.

[5] Heidelberger, M., and Svedberg, T., *Science*, 1934, **80**, 414.

[6] Heidelberger, M., and Pedersen, K., *J. Gen. Physiol.*, 1935, **19**, 95.

8394 C

# Growth of Human Nervous System. I. Growth of Cerebral Surface.

MEREDITH B. HESDORFFER AND RICHARD E. SCAMMON.

*From the Graduate Faculty and the Institute of Child Welfare, University of Minnesota.*

The interest in the extent and variability of the cortex of the human brain has led to a number of estimates of the surface area of the cerebrum (generally in the adult) by a variety of ingenious methods.[1-7] We have attempted to extend these studies by an experimental investigation of this area through a portion of the developmental period (from the fourth fetal or lunar month of prenatal life to 2 postnatal years) and in maturity. Our technique is described in detail in a forthcoming paper.[8] Briefly stated, the method consists of sectioning formalin fixed brains enclosed in a matrix with a mechanical device into slices 2 to 3.5 mm. in thickness. The area is then determined by measuring the outline of each section with a chartometer and multiplying the reading by the thickness of the section. The surface of the cerebrum is approximated by the sum of the values so obtained. Attempts to improve this technique by computing the sections as segments of cones and taking the means of their anterior and posterior outlines did not increase the accuracy of our determinations. Twenty cerebri were so studied. Figure 1 shows 10 of these drawn to scale (left lateral views) to illustrate the changes in size and form and in the configuration of the sulci in the series.

In measuring these structures figures were obtained for both "total" and "free" surface. "Total" surface indicates the entire cerebral surface including that portion buried in all of the cerebral fissures regardless of their depth. "Free" surface is a term used for the visible or external surface of the cerebrum only. In determining this value the chartometer was passed around the periphery of each

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1 Aresu, M., *Arch. Ital. Anat. e Embriol.*, 1914, **12**, 380.

2 Henneberg, R., *J. Psychol. u. Neurol.*, 1910, **17**, 144.

3 Kraus, W. M., Davison, C., and Weil, A., *Arch. Neurol. and Psychiat.*, 1928, **19**, 454.

4 Kraus, W. M., and Ditto, M. W., *Arch. Neurol. and Psychiat.*, 1927, **17**, 193.

5 Leboucq, G., *Compt. Rend. l'Ass. d. Anat.*, 1926, **21**, 338.

6 Paulier, A. B., *Compt. Rend. Soc. Biol., Paris*, 1892, Ser. 9, **3**, 133.

7 Wagner, H., *Diss. Göttingen*, 1864.

8 Hesdorffer, M. B., and Scammon, R. E., *Anat. Record* (accepted for publication), 1936, **64**.

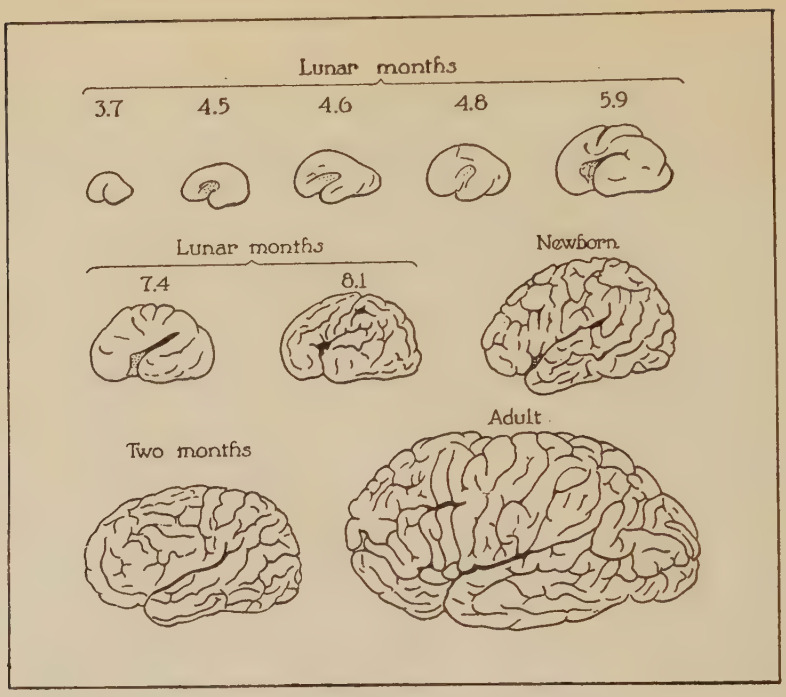


FIG. 1.
Ten outline drawings of the left lateral surfaces of human cerebri used in this study. All drawings made to absolute scale.

section but only dipped into the lips of the sulci to the shallow point where their sides meet. The medial surfaces of the cerebri were included in both types of measurement, but with the exclusion in both instances of the connecting masses of tissue such as the corpus callosum, the basal nuclei, the surface of the third ventricle, and the like. The results of these measurements are shown in Table I and Fig. 2.

There seem to be 3 stages in the growth with respect to time of "total" cerebral surface. Apparently there is a very rapid increase in the fourth lunar month, although we think our material is too limited to indicate any great or even regular increase in the fifth or sixth month. In the seventh and eighth lunar months, however, there is evidently a very vigorous increase in total surface of the cerebrum and there is obviously an even greater growth between 8 lunar months and birth.

In postnatal life there seems to be a marked absolute, although not relative, increase in infancy and very early childhood, but there is comparatively little, if any, increase after the latter period. The

TABLE I.
Observations of Human Cerebral Surface.

| Age | Cerebral Volume (cc.) | Cerebral Surface*—"Total" (sq. cm.) | Cerebral Surface*—"Free" (sq. cm.) | Cerebral Length (cm.) |
|---|---|---|---|---|
| (1) 3.7 lunar months† | 5.2 | 19.7 | 15.4 | 2.5 |
| (2) 4.5 " " | 22.5 | 58.9 | 47.1 | 4.8 |
| (3) 4.6 " " | 14.7 | 39.7 | 33.2 | 3.8 |
| (4) 4.7 " " | 18.7 | 49.2 | 43.2 | 4.5 |
| (5) 4.8 " " | 22.8 | 45.0 | 38.2 | 4.5 |
| (6) 4.8 " " | 22.0 | 49.8 | 41.7 | 4.1 |
| (7) 5.9 " " | 57.0 | 105.2 | 77.6 | 6.5 |
| (8) 7.2 " " | 114 | 184.2 | 126.6 | 7.2 |
| (9) 7.4 " " | 87.0 | 128.2 | 93.1 | 7.0 |
| (10) 8.1 " " | 106 | 176.0 | 105.8 | 8.0 |
| (11) Newborn | 320 | 679.3 | 219.9 | 11.0 |
| (12) " | 340 | 716.2 | 241.2 | 10.2 |
| (13) 0.17 yr. | 375 | 724.1 | 249.9 | 11.0 |
| (14) 0.32 " | 455 | 954.1 | 333.2 | 13.3 |
| (15) 0.44 " | 440 | 944.1 | 294.8 | 11.8 |
| (16) 2 " | 970 | 1666.4 | 457.3 | 15.6 |
| (17) 26 " | 1000 | 1635.3 | 552.9 | 17.2 |
| (18) 44 " | 1025 | 1610.1 | 568.7 | 17.2 |
| (19) 49 " | 955 | 1468.7 | 523.1 | 16.5 |
| (20) Adult (age unknown) | 795 | 1437.2 | 494.0 | 17.0 |

*Cerebral surface calculated to 2 decimals and thrown to one.
†Ages of fetuses calculated from body length by the Scammon-Calkins ('29) empirical formula.

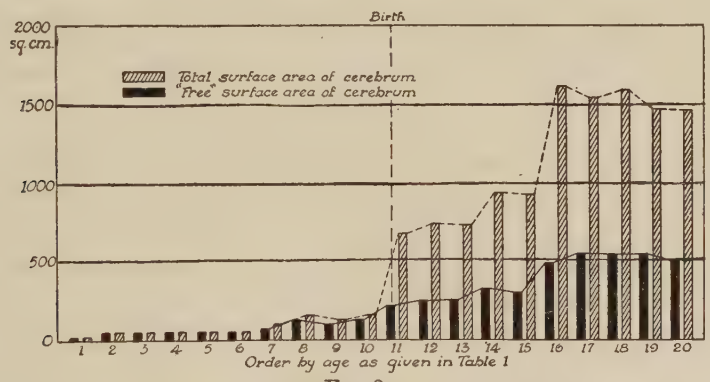


FIG. 2.

A bar diagram illustrating the growth of the "total" and "free" surface of the cerebrum. The shaded bars represent "total" cerebral surfaces and the black bars the concomitant "free" cerebral surfaces. The bars are arranged in order of age, but no attempt has been made to space them with respect to time.

"total" surfaces of the cerebrum more than double in postnatal life, but our limited set of observations (assuming that case No. 16 is normal, as all objective evidence indicates) is a process confined to early postnatal life.

The "free" cerebral surface follows much the same course as "total" surface until about 8 lunar months, but the increase between

this time and two years, while striking, is much less rapid. As in the case of "total" surface, the relative increase is small.

We may, then, regard the interval from about 6 lunar months to some time in the second postnatal year as the period of rapid absolute increase in cerebral surface, with a peak of the most noticeable growth in "total" surface area in the last trimeter of fetal life. These observations seem to be in accord with earlier purely morphologic observations on the time and extent of formation of the cerebral sulci in man. Our figures indicate some decrease in cerebral surface after the third decade, but again we do not think the series sufficiently large to warrant this assumption.

The relationships of cerebral volume and length to cerebral surface, which seem to us the more interesting, will be considered in following papers.

8395 C

## Growth of Human Nervous System. II. Indices of Relation of Cerebral Volume to Surface in Developmental Period.

RICHARD E. SCAMMON AND MEREDITH B. HESDORFFER.

*From the Graduate Faculty and the Institute of Child Welfare, University of Minnesota.*

The estimation of the area of the human cerebrum has become a matter of considerable interest since the mass of the cerebral cortex is closely related to the surface area of the brain.

This subject has been investigated by a quantitative study of 20 brains ranging in age from the fourth (lunar) month of prenatal life to the close of the fifth decade and in volume from about 5 cc. to over 1000 cc. The method of measuring surface area is described in other papers[1, 2] and the volume was determined by the displacement method. Various indices of the relation of cerebral volume to surface are shown in Table I and in Fig. 1. In both the table and the figure the observations are arranged in order of cerebral volume.

Column (b) of the table and panel (A) of Fig. 1 show the index of "total" surface area divided by cerebral volume (surface in sq. cm., volume in cc.). The index drops slowly at first, until the cere-

[1] Hesdorffer, M. B., and Scammon, R. E., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 415.

[2] Hesdorffer, M. B., and Scammon, R. E., *Anat. Rec.*, 1936, **64**, in press.

TABLE I.
Cerebral Indices in Prenatal and Postnatal Life.

| Order by Cerebral Volume | Arithmetic | | Geometric | | "Free" to Spherical "Total" Surface | | Age |
|---|---|---|---|---|---|---|---|
| | $\frac{S}{V}$ | $\frac{FS}{V}$ | $\frac{S}{V^{2/3}}$ | $\frac{FS}{V^{2/3}}$ | $\frac{S}{S'}$ | $\frac{FS}{S} \times 100$ | |
| (a) | (b) | (c) | (d) | (e) | (f) | (g) | (h) |
| 1 | 3.80 | 2.96 | 6.57 | 5.12 | 1.36 | 77.9 | 3.7 lunar months |
| 2 | 2.70 | 2.26 | 6.61 | 5.53 | 1.37 | 83.7 | 4.6 " " |
| 3 | 2.63 | 2.31 | 6.97 | 6.11 | 1.44 | 87.7 | 4.7 " " |
| 4 | 2.27 | 1.90 | 6.35 | 5.31 | 1.31 | 83.6 | 4.8 " " |
| 5 | 2.62 | 2.10 | 7.38 | 5.90 | 1.53 | 80.0 | 4.5 " " |
| 6 | 1.97 | 1.67 | 5.59 | 4.74 | 1.16 | 84.8 | 4.8 " " |
| 7 | 1.85 | 1.36 | 7.09 | 5.22 | 1.47 | 73.6 | 5.9 " " |
| 8 | 1.47 | 1.07 | 6.53 | 4.74 | 1.35 | 72.6 | 7.4 " " |
| 9 | 1.66 | 1.00 | 7.85 | 4.72 | 1.62 | 60.2 | 8.1 " " |
| 10 | 1.62 | 1.11 | 7.78 | 5.34 | 1.62 | 68.7 | 7.2 " " |
| 11 | 2.12 | 0.69 | 14.51 | 4.70 | 3.00 | 32.4 | Newborn |
| 12 | 2.11 | 0.71 | 14.70 | 4.95 | 3.04 | 33.7 | " |
| 13 | 1.93 | 0.66 | 13.92 | 4.80 | 2.88 | 34.5 | 0.17 years |
| 14 | 2.08 | 0.72 | 16.49 | 5.76 | 3.34 | 34.9 | 0.32 " |
| 15 | 2.17 | 0.68 | 15.95 | 4.98 | 3.38 | 31.2 | 0.44 " |
| 16 | 1.81 | 0.62 | 16.74 | 5.75 | 3.46 | 34.4 | Adult (age unknown) |
| 17 | 1.64 | 0.58 | 15.14 | 5.39 | 3.13 | 35.6 | 49 years |
| 18 | 1.57 | 0.43 | 17.01 | 4.66 | 3.52 | 27.4 | 2 " |
| 19 | 1.54 | 0.52 | 16.35 | 5.53 | 3.38 | 33.8 | 26 " |
| 20 | 1.72 | 0.61 | 15.80 | 5.58 | 3.27 | 35.3 | 44 " |

Explanatory note: Ages of fetuses calculated from body length by the Scammon-Calkins ('29) empirical formula. S = observed "total" cerebral surface; V = observed cerebral volume; FS = observed "free" cerebral surface; S′ = calculated surface of a sphere equal in volume to observed volume of corresponding cerebrum. Broken line in (E) indicates mean value of observed indices. Dotted line in (E) indicates calculated value of ratio of surfaces and volumes of spheres equivalent to observed volumes of cerebri.

brum has a volume of nearly 100 cc. (in the eighth lunar month), and shows practically no regular trend of change thereafter.

A better index is the "total" surface of the cerebrum divided by the two-thirds power of the cerebral volume, for this measure considers the factor of dimensionality. The index thus determined [Table I, column (d), Fig. 1, panel (B)] shows no prominent change until the cerebrum attains a volume of nearly 100 cc. (in the eighth fetal or lunar month) and rises abruptly to a new plateau at a period (just before birth) when there is relatively little increase in volume, and thereafter shows little significant change. There could hardly be a better demonstration of the great relative increase in cerebral surface area by fissuration in later fetal life.

Column (f) and panel (C) show indices of the "total" surface area divided by a value called S′, which is the geometrically calculated surface of spheres of volumes equal to those of the corresponding observed cerebri. Obviously the picture is much the same as

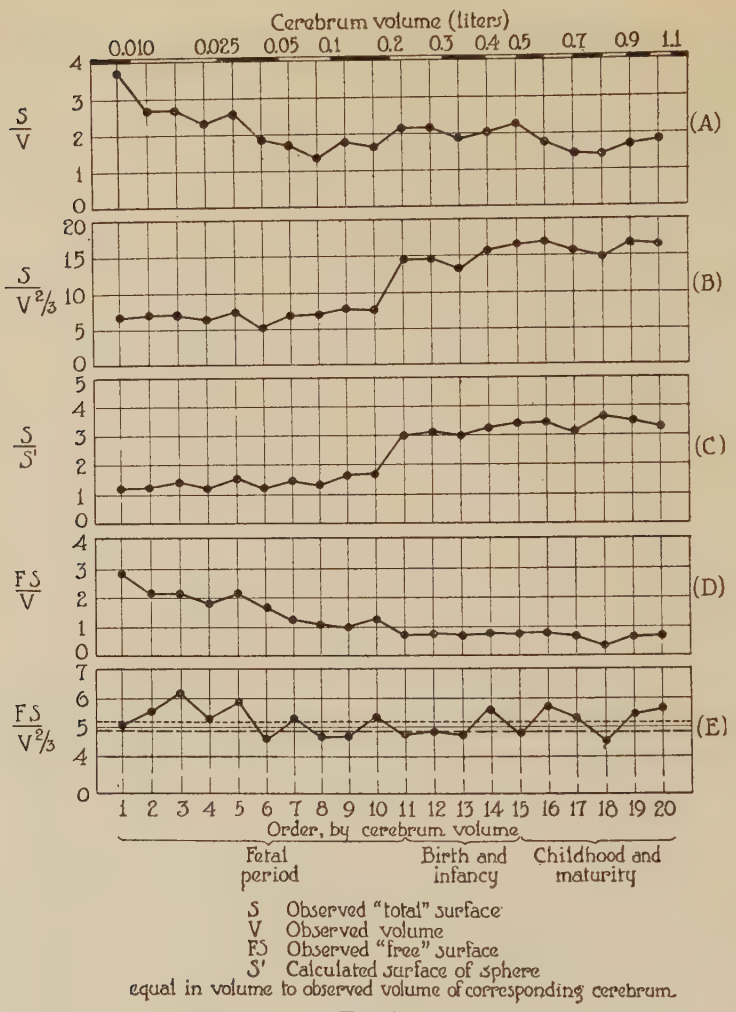


FIG. 1.
Indices of the growth of cerebral surface and volume in man.

in (B), although the numbers and irregularities in the values shown on the graph are smoothed by use of the fractional exponent in the denominator.

Column (c) and panel (D) in Fig. 1 show the relation of the "free" surface of the cerebrum to cerebral volume. Here, as in (A), we see a gradual decline in the index until the cerebrum attains a volume of somewhat over 300 cc. (at birth) and little change thereafter.

Column (e) and the lowest panel (E) in Fig. 1 show the index of the "free" surface of the cerebrum to the two-thirds power of its

volume, again a ratio which is theoretically correct dimensionally. The index so calculated shows no significant change in the series of observations (from a cerebrum having a volume of 5.2 cc. to one with a volume of over 1600 cc.). The values fluctuate, probably with the intrinsic variability of the material and technical inaccuracies of determining cerebral surface and volume. The upper, broken line on this panel of the graph is the mean for the entire number of observed indices in this series (5.2); the lower line is the ratio (R) of the surface of a sphere to the two-thirds power of its volume. This is,

$$R = \frac{4\pi r^2}{(4/3\,\pi r^3)^{2/3}} = 3^{2/3} \cdot (4\pi)^{1/3} = 4.84$$

The closeness of these calculated and observed ratios seems to us very striking and hardly to be accounted for by chance.

Finally, the relation between the "free" and the "total" cerebral surface was determined in the form of a ratio by dividing the former by the latter. The figures thus obtained are shown in Table I, column (g). In general "free" surface is equal to between four-fifths and two-thirds of the "total" surface until the cerebrum attains a volume of the order of 100 cc. (in the eighth fetal month). The ratio then drops rapidly as fissuration takes place until birth, and in postnatal life the "free" surface is approximately one-third of the "total" surface, regardless of the cerebral size.

# Illinois Section

*University of Illinois Medical School, November 26, 1935.*

## 8396 P

### Antiscorbutic Properties of Methyl-2-Keto-Gluconate.

A. E. SIEHRS, PAUL GOTTARDO, F. G. BRAZDA AND C. O. MILLER.
(Introduced by C. J. Farmer.)

*From the Department of Physiological Chemistry, Northwestern University Medical School.*

The antiscorbutic properties of various substances related structurally to ascorbic acid have been investigated and reported. We have investigated the antiscorbutic properties of methyl-2-keto-gluconate.

Sixteen guinea pigs, between 250 and 300 gm. in weight, were placed on a modified Dutcher scorbutic diet[1] consisting of rolled oats, white wheat flour, and alfalfa flour. They were fed 3 cc. of orange juice daily for 7 days before the start of the test experiment. During this time they showed a constant daily gain in weight. They were divided into 6 groups.

Group 1. Three animals. All showed loss of weight in 10-15 days, followed by the appearance of scorbutic symptoms. One was fed 3 cc. of orange juice at the 23rd day, but died of scurvy at the 26th day. The other 2 were fed 50 mg. and 70 mg. daily of methyl-2-keto-gluconate starting at the 23rd day, after which they quickly gained weight, lost their scorbutic symptoms, and grew steadily. At the 41st day the supplement was removed, and they gradually lost weight, developed scurvy, and died at the 53rd and 55th day, respectively.

Group 2. Two animals. Began to lose weight at the 10th day, and had developed gross scorbutic symptoms by the 15th and 16th days. On the 20th day one animal was started on 100 mg. of supplement daily, but died on the 23rd day. The other was given 70 mg. of supplement daily starting at the 20th day. At the 27th day this was increased to 100 mg., and the animal began to gain

[1] Dutcher, *Ind. Eng. Chem.*, 1924, **16**, 1070.

weight. At the 36th day, when the animal was in good condition, the dose was cut down to 50 mg. daily, and finally discontinued at the 41st day. Scorbutic symptoms developed on the 44th day, and the animal died on the 52nd day.

Group 3. Two animals. Fed 20 mg. of supplement daily until the 38th day, during which time they gained steadily in weight and were in good condition. After removal of supplement, they developed scorbutic symptoms at the 45th day and died of scurvy at the 59th and 60th days.

Group 3. Two animals. Fed 40 mg. of supplement daily to the 38th day and were in good condition and gaining weight. Following removal they developed scorbutic symptoms on the 47th and 50th days, and died of scurvy on the 55th and 60th days, respectively.

Group 4. Two animals. Fed 50 mg. of supplement and gained weight satisfactorily. On the 47th day supplement was removed, and they developed scorbutic symptoms on the 57th day, and died of scurvy at the 66th and 69th days.

Group 5. Two animals. Fed 70 mg. of supplement daily. One showed a satisfactory gain in weight, then became ill and lost weight, and died in 19 days. Autopsy showed no gross pathological signs of scurvy. The other animal gained weight steadily, and the supplement was removed at the 49th day. The animal developed scorbutic symptoms on the 87th day and died on the 103rd day.

Group 6. Two animals. Fed 100 mg. of supplement daily. Both animals gained weight satisfactorily. Supplement was removed at the 47th day, and gross scorbutic symptoms developed at the 51st and 57th days. The animals died of scurvy at the 59th and 74th days, respectively.

In another feeding experiment, one animal was fed the Dutcher diet plus a daily supplement of 70 mg. of the methyl ester for 33 days, during which time it gained weight satisfactorily. The supplement was then removed, and at the 40th day the animal began to lose weight and develop gross scorbutic symptoms. On the 45th day, supplement feeding was resumed, and the animal soon recovered, showing no scorbutic symptoms, gained weight constantly, and at the 110th day was still free of scorbutic symptoms, and was still gaining weight.

Our experiments show that methyl-2-keto-gluconate protects guinea pigs against scurvy when fed at the levels of 20, 40, 50, 70 and 100 mg. per day. In 4 animals it acted curatively in doses of 50, 70, and 100 mg. per day.

8397 C

## Relation of Dietary Fats to Action of Thyroid Extract in Rats.

S. LOUMOS. (Introduced by A. C. Ivy.)

*From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.*

In a previous paper,[1] the relation of some fats, namely, cod liver oil, olive oil, and cocoanut oil, to the action of thyroid extract in rats was studied. It was found that when normal rats are fed thyroid extract and olive oil, the latter at first decreases the rate at which weight is lost. However, if the olive oil is given after considerable loss of weight has occurred, the rate of loss of weight is augmented. Cod liver oil was found to be definitely antagonistic to the loss of weight when given at any period. Cocoanut oil showed no antagonistic action and increased weight loss when administered during any stage of hyperthyroidism. In this paper the results obtained from a study of 2 other dietary fats, namely, lard and crisco will be reported.

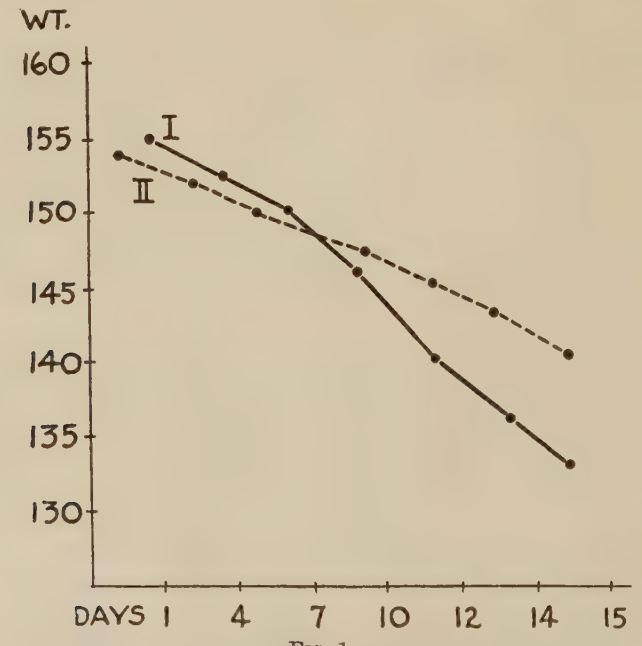


Fig. 1.

Simultaneous feeding of thyroid extract and lard. Curve I is the weight curve of the thyroid extract-fed control. Curve II of the litter mate receiving thyroid plus lard.

[1] Loumos, Proc. Soc. Exp. Biol. and Med., 1934, **31**, 895.

The method used in the previous experiments was employed. The animals were fed the standard diet and kept in individual cages. One hundred milligrams of thyroid extract (U.S.P.) were added to the diet. The control rats received the diet plus thyroid extract, water being used to make a paste. The treated rats received the diet plus thyroid extract plus the fat to be tested, the caloric intake being kept constant in all experiments. Litter mates were distributed between the control and treated groups.

*Thyroid extract and pure lard.* Twenty-four "adult" rats (150-165 gm.) were used. The 12 rats which received the lard (3 cc. daily) plus thyroid extract, lost weight less rapidly during the first 2 weeks than the control rats. The action of lard was quite similar to olive oil. (Fig. 1.)

*Thyroid extract and crisco.* The experiment was repeated in 24 "adult" rats. The results were analogous to those obtained with cocoanut oil. The addition of this fat to the diet very definitely augmented the action of thyroid extract after the first few days. The characteristic preliminary protective effect of olive oil and pure lard was not observed. This is shown in Fig. 2.

(The significance of and the literature related to these observations were discussed in the previous paper.)

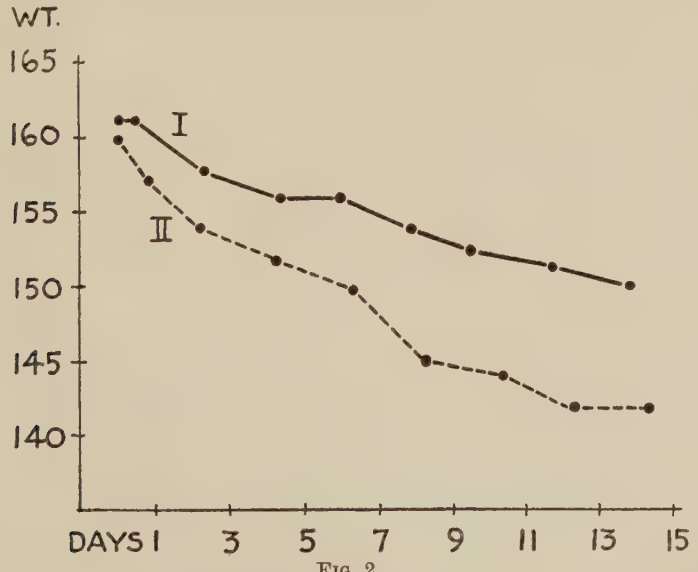


FIG. 2.

Simultaneous feeding of thyroid extract and crisco. Curve I is the weight curve of the thyroid extract-fed control. Curve II of the litter mate receiving thyroid plus crisco.

*Conclusion.* All fats do not bear the same relation to thyroid extract action. Some fats as cod liver oil, olive oil, lard, antagonize to different degrees the action of thyroid extract; others, as cocoanut oil, crisco, do not.

8398 C

## Homologous (Resonance-like) Function in Supernumerary Fingers in a Human Case.

PAUL WEISS.

*From the Department of Zoology, The University of Chicago.*

In amphibia, a supernumerary muscle during spontaneous and reflex activity contracts simultaneously with the homologous normal muscle of the same name.[1] Since this is true for each individual muscle, a supernumerary limb (or limb fragment) always exhibits the same movements as the nearby normal limb (or corresponding limb fragment) of the same side.[2] From this phenomenon has been derived the concept of a "resonance-like" principle of communication in the nervous system.[3] The experimental evidence has been confined, thus far, to the amphibia, where supernumerary limbs can be produced at will. In order to test the validity of the principle of homologous ("resonance-like") response in higher vertebrates and man, where the transplantation of supernumerary limbs has not yet been possible, we must avail ourselves of those cases in which congenital duplications of limbs or of some of their parts present us with a comparable situation. A human case favorable to such study has recently been described by Halverson and Amatruda,[4] and was re-examined by the author as to its bearing on the problem of homologous function.

The case concerns a girl, L. S., 14 years of age, with congenital duplication of the left forearm and hand. While the radial part of the hand, including the thumb, is absent, its place is taken by a mirror image of the normal ulnar part. The resulting double formation is a symmetrical hand with what appears to be the original

[1] Weiss, P., *Pflüger's Arch. f. d. ges. Physiol.*, 1931, **226**, 600.

[2] Weiss, P., *Roux' Arch. f. Entwmech.*, 1924, **102**, 635.

[3] Weiss, P., *Ergebn. d. Biol.*, 1928, **3**, 1. See summary in P. Weiss, *J. Comp. Neur.*, 1935, **61**, 135.

[4] Halverson, H. M., and Amatruda, C. S., *J. General Psychol.*, 1935, **13**, 140.

index as axis of symmetry and 2 groups of 3 fingers each to either side of this index. The order of the supernumerary fingers being reversed, the 7 fingers of the hand can be identified, beginning with the most lateral one, as (n stands for normal, e for extra fingers) : n5, n4, n3, n2, e3, e4, e5. The supernumerary fingers are well formed but somewhat weaker than the corresponding normal fingers; the anatomical details and general observations concerning the functioning of the member can be found in the report of Halverson and Amatruda.

A re-examination of the voluntary and reflex movements of the fingers at once revealed the validity of the principle of homologous function in this case. Corresponding n- and e- fingers moved in unison, identical muscle groups contracting simultaneously. n2 can be moved independently of the rest. n3 and e3 always work together, and so do n4+n5 and e4+e5 (a dissociation between 4 and 5 has never been clearly observed so that these 2 must be rated as one). When L. was told to clutch a rod or the author's finger with her normal 3rd, 4th, and 5th fingers, the supernumerary 3rd, 4th, and 5th performed the same flexion in the air. When the 3rd was left extended, the supernumerary 3rd also failed to flex. When, after she had taken a firm grip on the rod, she was told to extend the 3 supernumerary fingers, this could not be done without the 3 corresponding normal fingers losing their grip and extending also.

In the absence of special recording apparatus, the following procedure was adopted as the most objective method of testing homology of response: The author had L. clutch one of his hands with her 3 extra fingers and held in his other hand the corresponding normal fingers which were equally flexed. L. was told to resist the passive stretching of any of her fingers by the author. If the author, then, tried to extend, for instance, n3, thus evoking an increased effort to keep this finger flexed, immediately an increased pressure was felt to be exerted by the corresponding e3, which had been quietly resting in the author's other hand. If passive extension was attempted on n4+n5, pressure by e4+e5 was felt to increase correspondingly. In this test the muscular contractions were chiefly isometrical and concealed from the subject.

When L. was ordered to tap the table with n3, keeping all the other fingers quiet, e3 invariably accompanied n3 in the tapping movement, while the other fingers, including the index separating n3 from e3, remained fairly inactive. Abduction of n3 from n2 could not be effected without simultaneous abduction of e3; since

n3 and e3 are mirror images of each other and their abductors lie on different sides, n3 and e3 move in opposite directions, either both away from the central index or both toward it, which clearly proves that the phenomenon is a matter of homologous function and not the result of mechanical association through common tendons.

In all the reported experiments, L. was, of course, prevented from controlling her movements by vision. In addition, the tests taken were absolutely new to her and she did not realize their purpose. Her low I.Q. (70-75) was a welcome safeguard against intentional deception on her part.

In general, we may state then that, concomitant with every movement of the normal part of the hand, a corresponding symmetrical movement appears in the supernumerary part. Only in one instance could a disturbance of this essential symmetry be observed: L., after grasping an object with e3, e4, and e5, whereby n3, n4, and n5 also went into flexion, was told to make efforts to extend n3-5 while leaving e3-5 bent. Under visual control, she could manage, after several futile attempts, to bring n3-5 into some sort of cramp-like extension without corresponding extension of e3-5. However, neither did the flexors of n3-5 relax completely, nor did the flexors of e3-5 remain in their previous vigorous flexion. As soon as the efforts to extend n3-5 were made, the grip of e3-5 lost its power, revealing that although e3-5 did not actually become extended, some reciprocal inhibition of the flexors, which forms part of every extension, had appeared. The residual flexor contraction, present equally in n3-5 and e3-5, was overbalanced, however, in the former group by an extensor contraction for which there was no noticeable counterpart in the latter. Since the flexor muscles of the two sets were found to retain their functional association, and also because in the reciprocal test (see above) independent extension of e3-5 could not be obtained, it is very likely that the apparently independent extension of n3-5 does not represent a real dissociation between 2 sets of homologous muscles. Presumably, the explanation lies in some asymmetry in the development of the long dorsal extensor muscles, some of which may be present in the normal part, absent in the abnormal part of the arm. The external aspect of the hand seems to support this view, although no decision can be reached without a thorough study of the musculature. As an alternative explanation, one might feel tempted to contend that cortical activity (conscious effort) might possibly be able to annul the close association which exists on the spinal level between iden-

tical muscles of the same side. In order to test this possibility, it has been suggested to L. that she continue in her efforts to train the 2 parts of her left hand to independent action. It is intended to re-examine her on later occasion and to ascertain the extent to which her efforts may have been successful. The fact, however, that no independence and dissociation of the 2 parts has developed during the past 14 years of her life, makes it appear rather doubtful that there will be much success in the future.

On the sensory side, the "homologous" function, appearing as "homologous" feeling, was already evident to Halverson and Amatruda in their examination of the case. We can only reiterate that L., without visual control, identifies the supernumerary fingers e3, e4, e5, when touched individually, as middle finger, ring finger, little finger, respectively, (identification by pointing to the finger on her normal right hand which feels like the stimulated one). There is then a definite perception of the organ quality of a finger, irrespective of its place on the hand. This perception is probably mediated by proprioceptive nerves for which it has been demonstrated that something muscle by muscle specific is inherent in their excitations, enabling the centers to identify, and discriminate between, various muscles irrespective of their place.[5] While confounding corresponding fingers of her left hand with respect to their organ quality, L. was yet able to discern whether a stimulated finger was the farther lateral or the farther mesial of the particular pair of identical fingers present. It is most likely that this discrimination was effected through cutaneous stimuli the local signs of which, contrary to the proprioceptive sense, are probably not perceived by means of different constitutional specificities.[6]

In conclusion, this case proves convincingly: that the resonance-like mechanism of nervous communication which makes a given muscle (or rather the nerve specified by the muscle[7]), and at the same time all supernumerary identical muscles, respond whenever an impulse specific for that muscle is generated in the corresponding central district, holds for man as well as for amphibia, hence probably for the whole group of vertebrates; furthermore, that proprioceptive sensations are, in man as well as in amphibia, identified by some specificity of the discharges of the sensory organs (or rather of the proprioceptive nerves specified by them), rather than by local

[5] Verzar, F., and Weiss, P., *Pflüger's Arch. f. d. ges. Physiol.*, 1930, **223**, 671. Weiss, P., *Pflüger's Arch.*, 1931, **228**, 486.

[6] Weiss, P., *Wiener Klin. Wchschr.*, 1931, **39**, 13.

[7] Weiss, P., *Anat. Rec.*, 1934, **60**, Suppl., 30.

signs. Brain activity, guided by sensory control, can in higher vertebrates change the pattern of coordination; but the human case described above does not encourage the view that it can also break down the unfailing obedience of a muscle (or its nerve) to its appropriate central discharges, as would be required in order to dissociate between 2 identical muscles innervated from the same level and the same side of the cord.

8399 P

## Alterations in Serum Proteins as an Index of Liver Failure.

EDMUND F. FOLEY, ROBERT W. KEETON, AARON B. KENDRICK AND DUANE DARLING.

*From the Department of Medicine, University of Illinois, College of Medicine.*

For the past two years we have been interested in the study of the blood proteins in known or suspected cases of liver injury. That there is an alteration in the blood proteins in liver injury has been known since Glenet[1] showed a reduction of the total protein and numerous studies established that there was an elevation of the serum globulin (Felinski[2]) and a reversal of the albumin globulin ratio in cirrhosis of the liver with ascites (Abrami and Wallach[3]). Wiener and Wiener[4] obtained a reversal of the A/G ratio in cirrhosis and a hyperglobulinemia in acute infections. Meyers and Keefer[5] and Snell,[6] in cases of decompensated cirrhosis could not attribute the depletion of the serum albumin to its loss in the ascitic fluid.

Serum albumin, serum globulin and total serum protein determinations were made according to the hypobromite gasometric method of Van Slyke in cases of decompensated cirrhosis, cases with choluric jaundice, both intrahepatic and extrahepatic, without ascites, cases of ascites without clinical cirrhosis, a miscellaneous contrast group as well as a control group of normals. These normals served as a check on our technique and established values comparable to the accepted normal values of Peters and Van Slyke.

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1 Grenet, *Mem. Soc. de Biol.*, 1907, **63**, 532.

2 Felinski, *La Presse Medicale*, 1922, **30**, 236.

3 Abrami, P., and Wallech, Robert, *Comp. Rendes Soc. de Biol.*, 1930, **101**, 291.

4 Wiener, H. J., and Wiener, R. E., *Arch. Int. Medicine*, 1930, **46**, 236.

5 Meyers, W. K., and Keefer, C. S., *Arch. Int. Medicine*, 1935, **55**, 349.

6 Snell, A. M., *Proc. Staff, Mayo Clinic*, 1935, **10**, 481.

In 21 cases of decompensated cirrhosis there was a constant hypoalbuminemia and a hyperglobulinemia with a consequent reversal of the A-G ratio, with an average ratio of 0.55 instead of the normal average of 2.48. In no case was paracentesis performed prior to the serum protein determinations. Of particular interest as evidence of the prognostic significance was the duration of life after the serum proteins had undergone these changes. In one instance, for example, in which the serum albumin was 2.3 g. the globulin 5.46, the A-G ratio 0.4, the duration of life was 5 days. The maximum duration of life with pronounced reversal was 70 days.

There were 3 cases of extrahepatic jaundice due to stone impacted in the common bile duct which showed an elevation of the globulin fraction with a corresponding reversal of the A-G ratio. In these cases in spite of careful preoperative care, conservative surgery and postoperative treatment including intravenous glucose and buffer solution, decholin and duodenal drainage, death occurred, while in a similar case in which there was no reversal the operation was followed by recovery. In malignant obstructive jaundice the serum protein findings were not striking though there was a tendency for the globulin to be elevated.

In severe intrahepatic jaundice there was a marked depletion of the serum albumin and elevation of the globulin with extreme reversal, while in 7 cases of catarrhal jaundice the protein values were approximately normal.

In 8 cases of ascites, due to carcinoma of the peritoneum, tuberculous peritonitis, congestive heart failure and in which cirrhosis was not clinically apparent, no reversal of the A-G ratio occurred. The contrast cases consisted of 3 cases of starvation in which there was a marked decrease in the protein intake. Low total blood proteins were obtained in these cases but with a normal ratio of albumin and globulin. Also included in this group were cases of hepatosplenomegaly and compensated cirrhosis of the liver and in these there was no significant change in the serum proteins.

From this study it would seem that in grave liver injury there is a disturbance of the blood proteins consisting of a lowering of the serum albumin and an elevation of the serum globulin with a reversal of the A-G ratio. This further offers clinical indication that the liver is an important site of serum albumin formation and that alterations in the serum proteins are an index of liver failure.

## 8400 C

# Ionized Blood Calcium in Patients with Renal Calculi.

HERBERT POLLACK AND MIRIAM REINER.

*From the Metabolic Clinic and the Division of Laboratories, The Mount Sinai Hospital, New York.*

The statement has been made that hyperparathyroidism is frequently found as the etiological factor in renal calculi.[1] It becomes of interest to examine this hypothesis by all possible methods at our disposal. Since one may find normal total calcium figures in the pres-

TABLE I.

| No. | Mg. P /100 cc. | Protein % (gm.) 100 cc. | Mg. Ca /100 cc. | Ionized Ca Mg. /100 cc. | Diagnosis |
|---|---|---|---|---|---|
| 1 | 3.81 | 6.36 | 8.63 | 4.00 | Renal calculus |
| 2 | ..... | 6.57 | 10.45 | 4.80 | " " |
| 3 | 2.86 | 7.12 | 10.40 | 4.60 | " " |
| 4 | ..... | 6.68 | 10.50 | 4.75 | " " |
| 5 | 3.80 | 5.90 | 9.50 | 4.75 | " " |
| 6 | 4.00 | 6.90 | 10.00 | 4.50 | " " |
| 7 | 3.20 | 6.95 | 10.00 | 4.45 | " " |
| 8 | 2.90 | 6.91 | 10.10 | 4.50 | " " |
| 9 | 3.80 | 6.92 | 10.45 | 4.6 | " " |
| 10 | 4.44 | 5.91 | 10.45 | 5.2 | " " |
| 11 | ..... | 7.12 | 9.84 | 4.3 | " " |
| 12 | 4.12 | 7.20 | 9.60 | 4.2 | " " |
| 13 | 4.44 | 7.06 | 10.77 | 4.7 | " " |
| 14 | 3.20 | 7.19 | 10.90 | 4.7 | " " |
| 15 | 2.65 | 7.50 | 9.90 | 4.25 | " " |
| 16 | 3.55 | 6.88 | 10.77 | 4.8 | " " |
| 17 | 3.90 | 7.44 | 10.23 | 4.3 | " " |
| 18 | 4.57 | 6.56 | 10.17 | 4.6 | " " |
| 19 | 3.90 | 6.38 | 10.66 | 5.1 | " " |
| 20 | 3.08 | 5.90 | 10.29 | 5.1 | " " |
| 21 | 3.08 | 6.70 | 11.03 | 5.1 | " " |
| 22 | 2.65 | 7.08 | 9.90 | 4.4 | " " |
| 23 | 3.63 | 7.10 | 10.78 | 4.7 | " " |
| 24 | 3.56 | 6.51 | 10.54 | 4.8 | " " |
| 25 | 3.48 | 7.96 | 10.77 | 4.4 | Diabetes |
| 26 | 3.08 | 6.78 | 9.92 | 4.5 | Control |
| 27 | 4.85 | 7.12 | 12.25 | 5.4 | " |
| 28 | 3.80 | 7.32 | 10.56 | 4.5 | " |
| 29 | 4.21 | 5.95 | 12.50 | 6.0 | Hyperthyroid |
| 30 | 5.16 | 6.74 | 10.70 | 4.85 | " |
| 31 | 3.50 | 6.53 | 8.50 | 3.85 | Subacute yellow atrophy of liver |
| 32 | 3.90 | 7.18 | 10.04 | 4.4 | Epilepsy |
| 33 | 4.10 | 7.14 | 10.10 | 4.4 | " |
| 34 | 4.20 | 6.43 | 10.80 | 5.1 | Hyperthyroid |
| 35 | 3.72 | 5.81 | 10.40 | 5.2 | Nephrotic syndrome |

[1] Albright, F., Baird, P. C., Cope, O., Bloomberg, E., *Am. J. Med. Sci.*, 1934, **187**, 49.

ence of hyperparathyroidism the simple determination of the total calcium and phosphorus is not entirely reliable.

McLean and Hastings[2] have recently proposed a simple method for the determination of the ionized calcium in the serum. This they believe is the most sensitive test available for detecting the presence of hyperparathyroidism.

Twenty-four patients with proven calcium stones were referred to the Metabolic Clinic by the Second Surgical Service (Dr. Edwin Beer). The Collip modification of the Tisdall method was used for the determination of the total serum calcium, and the Fiske-Subbarow method for the inorganic phosphorus. The serum proteins, after removal of non-protein nitrogenous substances, were determined by a modification of the Pregl micro-Kjeldahl procedure. The results are shown in Table I with the addition of data from control cases. These controls are used in addition to the normal figures published by McLean and Hastings.

There is no evidence of increased calcium ion concentration in any of the stone cases.

8401 C

## A Method of Purification of Gonad Stimulating Principle from Pregnant Mare Serum.

ARTHUR E. MEYER.

*From the Laboratory of the Chappel Foundation for Organo-Therapeutic Research, Rockford, Illinois.*

The preparation of extracts from pregnant mare serum presents a problem different from that found in the extraction of pituitary gland material. The main difficulty is found in the solubility of serum proteins which form colloidal solutions and cannot easily be separated from the gonad stimulating factor itself. Such solutions appear opalescent or turbid and pass through a bacteria-proof filter only at a very slow rate. The method as described by Evans, Gustus and Simpson[1] overcomes this difficulty by adsorbing the active principle on aluminum hydroxide. This method yields a highly purified product, but requires special equipment not available in every laboratory.

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2 McLean, F. C., Hastings, A. B., *Am. J. Med. Sci.*, 1935, **189**, 601.

1 Evans, Gustus and Simpson, *J. Exp. Med.*, 1933, **58**, 569.

Following is a rather simple method which gives a product suitable for biological and clinical use.

Serum from pregnant mares was precipitated in the usual way with 2 volumes of acetone, washed with acetone and dried. This precipitate formed the raw material used in the preparation of the extract. The powder was treated with a 6% solution of butyl alcohol in water, as described for pituitary gland extraction.[2] It is doubtful whether the addition of butanol, advantageous in the extraction of the pituitary gland, is of any particular value in this case, since the serum quickly swells up in water and does not present any problem as far as extraction is concerned. However, the slightly antiseptic properties of the butyl alcohol, which prevent putrefaction during the process of extraction at room temperature, made it desirable to apply this method to serum also.

One hundred grams of dry powder was extracted for at least 12 hours each time with 700, 700, 600, and 600 cc. of butanol solution. The combined extracting fluids were filtered and evaporated *in vacuo* to 500 cc. at a temperature below 40°. Four volumes of acetone were added, which caused a flocculent precipitate. After standing for 15 hours, the clear liquid was decanted from the precipitate. The latter was then extracted for 3 hours with 1 liter of water and 500 cc. of acetone, filtered and re-extracted with half the amount of the same mixture. The combined extracts were evaporated to 500 cc., resulting in a turbid aqueous solution (called crude extract) 5 cc. representing one gram of dry serum powder.

A concentrate solution of 5 gm. of crystallized aluminum sulfate was added under constant stirring, causing the formation of a heavy precipitate. The reaction of the supernatant liquid was pH 4.0 to 5. The material was difficult to filter, but this was overcome by adding 200 cc. of acetone. The precipitate was washed with 20% acetone, and the combined filtrate (about 700 cc.) was precipitated with 2 liters of acetone. After standing for 12 hours, the liquid could readily be poured off from the sediment. The latter was dissolved in 80 cc. of water, containing about ½ gm. of disodium phosphate. Practically all of the aluminum could be removed by careful addition of sodium hydroxide, adjusting the reaction closely to pH 7.0. A translucent solution was obtained by filtration. This was brought up to 100 cc., so that each cc. corresponded to 1 gm. of dry serum powder. The crude extract as well as the purified solution, was assayed on female rats 22 days old, as described (l. c.).

[2] Meyer and Fevold, PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 570.

TABLE I.

| | cc. injected | Gm. of serum powder contained | Wt. of ovaries* mg. |
|---|---|---|---|
| Crude | .05 | .01 | 15 |
| | .085 | .017 | 20 |
| Purified | .02 | .02 | 17 |
| | .029 | .029 | 22 |
| | .03 | .03 | 28 |

*Average of 4 rats.
Ovarian weight of the controls was 10 to 12 mg.

It can be estimated that about one-third of the potency of the material was lost, probably due to the quantity of liquid retained in the voluminous precipitate.

The residue from 1 cc. of the purified extract dried at 105° amounted to 10.7 mg. It consisted largely of sodium sulfate and phosphate. The loss by incineration was 2.6 mg., which figure represented the maximum of organic matter which might be present. As the above figures give an estimation of approximately 40 units per cc., it might be said that 1 mg. of organic substance represents the activity of 15 units.

Slow reprecipitation of the solution with acetone caused first the formation of a heavy precipitate containing practically all the inorganic material, but including most of the active principle. A fine layer of very light precipitate which did not settle out readily, could be separated. Only about 8 mg. of the latter could be collected from 200 gm. of serum powder. In the assay, an injection of 0.023 mg. produced ovaries of 16.3 mg. weight, opening of the vagina and stimulation of the uterus. The more impure and copious layer of inorganic salts included the larger part of the activity: 0.4 mg. increased the ovarian weight to 25.3 mg.

Removal of inorganic matter to a certain degree could be effected by transforming the sulfates into chlorides. While alkali sulfates are readily precipitated by adding 2 volumes of acetone to an aqueous solution, the chlorides remain dissolved. When serum powder of lower activity was used, as happens in slightly advanced stages of pregnancy, a larger quantity of aluminum sulfate was necessary. The sulfate content in the final product was increased accordingly. Transformation into chlorides was effected by addition of barium chloride, avoiding an excess of the latter; for that reason a trace of sulfate was allowed to remain.

The precipitate of barium sulfate was filtered off and the clear

liquid precipitated with 2 volumes of acetone. This precipitate was redissolved to the same volume as the previous solution. While the latter contained 5.26% solids with 1.52% apparent organic matter, the purified solution contained 1.40% solids and 0.63% organic matter. There was probably a certain loss through absorption, although the difference in organic matter must be explained in part by the loss of crystal water from the inorganic constituents. The assay showed 30 units per cc. in the unpurified, and 25 units in the purified product.

An attempt was made to apply the method to pituitary material, but results were not very satisfactory. By using isoelectric or benzoic acid precipitation for removal of proteins, horse pituitaries will give a solution containing from 70 to 100 units per gm. of dry gland material. When the aluminum sulfate method was used, only 35 units were found in the final product. Nevertheless, the quantity of organic material in the end product by the aluminum method was higher than in the extracts obtained by the other methods.

Sections from the ovaries* of the rats showed that luteinization was obtained with the pituitary as well as the serum extracts, as soon as quantities were given which caused an increase of at least 50% in ovarian weight. Luteinization was heavier with the pituitary material than with the serum extracts. When the serum extract was given at a level to produce less than a 50% increase in the weight of the ovaries, only follicle formation was observed. A separation of both gonad stimulating factors was obviously not obtained by this method.

* I am indebted to Dr. A. A. Hellbaum of the University of Wisconsin for the preparation of the sections from the ovaries.

8402 P

# Urinary Excretion of Ascorbic Acid in the Dog Following Ether Anesthesia.

DONALD E. BOWMAN AND EDWARD MUNTWYLER.

*From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland.*

Some time ago in a study in this laboratory[1] dealing with the influence of ether anesthesia on the acid-base balance in dogs it was noted that when silver nitrate was added to urine collected following the anesthesia for the chloride estimation, the silver was reduced and a black precipitate formed before the acid indicator was added. This observation led us to investigate the influence of the ether anesthesia on the excretion of ascorbic acid as determined by titration with 2:6-dichlorophenolindophenol.

Female dogs were employed. The urine was collected by catheterization into a bottle containing a few drops of glacial acetic acid, after which sufficient 20% trichloracetic acid was added to give a 5% trichloracetic acid concentration. This was then titrated into a known amount of 2:6-dichlorophenolindophenol. The dye was standardized with crystalline ascorbic acid (Merck) according to the procedure of Birch, Harris and Ray.[2] It was found, however, that when urine was allowed to stand even for short periods the reducing value decreased appreciably. Consequently it was thought necessary to perform a preliminary step of bubbling hydrogen sulfide through the urine for ten minutes and then allowing it to stand over night under an atmosphere of hydrogen sulfide, after which the $H_2S$ was driven off by bubbling nitrogen through the urine for 15 minutes (or until no nitroprusside test was obtained). The trichloracetic acid was then added and the titration carried out immediately.

Table I presents the results of one of a number of experiments which have been performed.

From these results it is observed that the urinary excretion of ascorbic acid as determined by titration with 2:6-dichlorophenolindophenol is markedly increased in the dog following ether anesthesia.

Recently Zilva[3] observed that when ether was used as a general anesthetic for the intravenous injection of l-ascorbic acid in guinea

[1] Pomerene, E., The Total Acid-Base Equilibrium in Anesthesia, Doctor's Dissertation, Western Reserve University, 1929.

[2] Birch, T. W., Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 590.

[3] Zilva, S. S., *Biochem. J.*, 1935, **29**, 2366.

TABLE I.
The Urinary Ascorbic Acid Following Ether Anesthesia.

| Date | Urine Volume cc. | Ascorbic Acid mg. per 100 cc. | Ascorbic Acid mg. per 24 hr. | Remarks |
|---|---|---|---|---|
| 6/ 5/35 | 994 | 3.2 | 31.7 | Control |
| 6/ 6/35 | 635 | 3.9 | 24.5 | " |
| 6/ 7/35 | 1610 | 11.8 | 190.0 | Two hours ether anesthesia |
| 6/ 8/35 | 810 | 2.6 | 22.0 | |
| 6/ 9/35 | 830 | 3.2 | 26.5 | |
| 6/14/35 | 500 | 6.1 | 31.3 | Control |
| 6/15/35 | 2385 | 11.1 | 266.0 | Two hours ether anesthesia |
| 6/16/35 | 300 | 3.6 | 10.9 | |
| 6/17/35 | 610 | 3.6 | 22.2 | |
| 6/20/35 | 850 | 3.8 | 29.6 | Control |
| 6/21/35 | 1571 | 16.2 | 255.0 | Two hours ether anesthesia |
| 6/22/35 | 1090 | 2.7 | 29.2 | |
| 6/23/35 | 710 | 4.8 | 34.2 | |

pigs, the urinary excretion of the l-ascorbic acid was increased. Other experiments are in progress dealing with the metabolism of ascorbic acid in the dog and also experiments attempting to explain the action of ether anesthesia in causing the increased urinary excretion.

8403 P

## New Pharmacological Actions of Physostigmine.

THEODORE KOPPANYI, CHARLES R. LINEGAR AND JAMES M. DILLE.

*From the Department of Pharmacology and Materia Medica, Georgetown University School of Medicine, Washington, D. C.*

While investigating the peripheral action of barbiturates it was observed that in all experimental animals where the cardiac vagus response to weak faradic stimulation had been abolished by large doses of most barbiturates, and by moderate doses of amytal and pernoston, the administration of physostigmine salicylate in doses from 0.2 to 0.35 mg. per kilo by vein, caused usually no detectable spontaneous effect upon the heart rate or blood pressure. The injection of comparable doses of acetylcholine and pilocarpine produced in the same animals a marked fall in blood pressure which in many cases was accompanied by a slowing of the heart.[1] If 2 or 3 minutes were allowed to elapse after the intravenous administration of physostigmine and then the peripheral vagus was stimulated elec-

[1] Koppanyi, Linegar and Dille, *Science*, 1935, **82**, 228.

trically, profound cardiac slowing and fall in blood pressure was produced in 6 dogs, 4 cats and 6 rabbits. In 7 experiments these vagus effects outlasted for several minutes the actual stimulation of the nerve. This physostigmine sensitization of the vagus to stimulation of the preganglionic fibers lasted for about a half hour and was antagonized in ten animals by further doses of barbiturates. Since barbiturates abolished the cardiac vagus response to faradic stimulation but not to pilocarpine, it was postulated that their vagus-impairing effects are due to ganglionic depression (see also Kobacker and Rigler,[2] and Garry.[3]) We have, therefore, employed nicotine and curare, drugs known to produce ganglionic paralysis. After sufficient doses of nicotine (2 to 10 mg. per kilo) or curare (3 to 6 mg. per kilo) had been given intravenously to abolish the peripheral vagus effects in 2 dogs, 3 rabbits and 3 cats, 0.2 to 0.3 mg. of physostigmine salicylate per kilo was injected intravenously. In every case within a few minutes following injection of physostigmine faradic stimulation of the peripheral vagus produced marked cardiac inhibition. Pilocarpine had no such effects. Therefore physostigmine antagonized the synaptic paralysis produced by nicotine and curare.

Since physostigmine abolished the block in parasympathetic synapses it was investigated whether it would act in a similar manner on sympathetic synapses. Nicotine and curare were employed intravenously, in the same doses as above, to abolish the eye effects (dilatation of the pupil, exophthalmos, withdrawal of the nictitating membrane and widening of the palpebral fissure) and the blood pressure responses which follow stimulation of the preganglionic fibers of the cervical sympathetic nerve. After sufficient doses of nicotine or curare were given to produce paralysis in the superior cervical ganglion, 0.2 to 0.3 mg. of physostigmine salicylate per kilo were injected intravenously in 3 cats and 2 rabbits. A few minutes after the injection, the preganglionic fibers of the cervical sympathetic were again stimulated and this was now followed by the characteristic ocular and vascular responses to sympathetic stimulation. Physostigmine, therefore, opposed the synaptic paralysis due to nicotine or curare in the sympathetic as well as the parasympathetic division.

Physostigmine does not sensitize the cardiac vagus to electrical stimulation if its excitability has been abolished by atropine (2 mg. per kilo).

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[2] Kobacker and Rigler, *J. Pharm. and Exp. Therap.*, 1930, **37**, 129.

[3] Garry, *J. Pharm. and Exp. Therap.*, 1930, **39**, 129.

It is possible that this newly discovered action of physostigmine might be due to inhibition of the esterase which is responsible for the destruction of acetylcholine and that in the final analysis the substance producing the ganglionic effect is acetylcholine. This interpretation of the effect on the sympathetic synapse seems to be in harmony with the view that the transmission through the sympathetic synapse is cholinergic (Feldberg and Gaddum[4]).

## 8404 C

## Procedure for Quantitative Extraction of Sex Hormones from Urine.

T. F. GALLAGHER, F. C. KOCH AND R. I. DORFMAN.*

*From the Department of Physiological Chemistry, The University of Chicago.*

The excretion of sex hormones in the urine has been the subject of a voluminous literature. Attempts have been made to relate the amount of these substances excreted to various pathological conditions with the hope that new information of clinical value would be made available. Unfortunately most of this effort is valueless because certain fundamental facts are completely ignored. Thus it is known from the researches of Zondek, Cohen and Marrian,[1, 2, 3] and others that some fraction of the estrogenic substance in urine is conjugated so that it is either not extractable, biologically inert, or both. Conversion to the active form, therefore, must be an essential feature of any method for assaying the urine for total content of female hormone. Further it is immediately evident that a unit expressed solely in terms of a biological response is almost useless for the purpose under discussion. The only unit acceptable is a definite quantity of substance. Such a standard for the female hormone has been available for several years and recently a standard has been designated for the male hormone. Unfortunately, few workers have used these or indeed any standards. Only by the use of such standards can the values obtained by a given individual and by different laboratories be accurately compared, for without a standard it is impossible to detect change in sensitivity of the test

[4] Feldberg and Gaddum, *J. Physiol.*, 1934, **81**, 305.

* This work was supported in part by a grant from the Rockefeller Foundation.

[1] Zondek, Bernhard, *Nature*, 1934, **133**, 209.

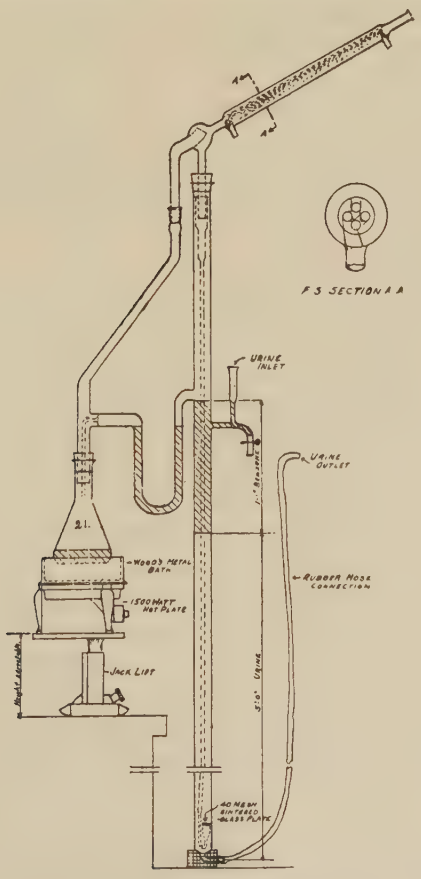
[2] Zondek, Bernhard, *Ark. Kemi., Min. o. Geol.*, 1934, part 3, paper 24.

[3] Cohen, S. L., and Marrian, Guy F., *Biochem. J.*, 1934, **28**, 1603.

animal, no matter what the cause. This assertion applies equally well for sex hormones or any biologically active substance. Finally, in order to assay normal urines it is necessary to extract the active substances. In order to accomplish this, one must be assured that the extraction is complete.

In the opinion of the authors, the only procedure fulfilling the necessary conditions is that of Cohen and Marrian. Unfortunately their technique is suitable only for urines of pregnancy, and as yet they have not extended their investigations to the male hormone. We, therefore, present the results of our investigations upon extraction and assay of the male and female components of normal urines.

Figure 1 shows the extraction apparatus we have used. By means of this device we are able to make as nearly complete contact as is




CONTINUOUS EXTRACTOR FOR SEX HORMONES FROM URINE

FIG. 1.

possible of the urine with an organic solvent and at the same time measure the rate of distillation without interrupting the extraction process. The rate of distillation achieved with benzene varies between 6 and 8 liters per hour. Thus, as we shall show, a 24-hour sample may be extracted in at most 2 hours. After some study, benzene was chosen as the most efficient solvent since it is most effective in removing both hormones with the minimum of extraneous substances. Toluene is equally as good but the higher boiling point is a disadvantage. Ethyl acetate, which has been used to some extent in this country, is relatively poor compared with benzene. It has, in addition, the disadvantage of dissolving a large amount of inert material which eventually may be toxic to the test animal.

All assays, both on spayed females and on capons, were conducted by assaying a standard preparation at the same time as the unknown. Ten rats and 7 capons were used on each assay reported here. For the estrogenic substance, the International Standard of the League of Nations' Committee was used. For the male hormone, we have used for 3 years a standard preparation which is exactly equal to 100γ of androsterone. We were fortunate inasmuch as this quantity has since been chosen as the international standard by the League of Nations' Committee.

In order to prepare the extract for assay the benzene was distilled, the residue dissolved in ethyl ether and the ethereal solution shaken with saturated aqueous sodium bicarbonate until reaction ceased. The bicarbonate removed only inert acids and was discarded. The ether solution was then worked through in either of 2 fashions. If sufficient urine had been extracted to allow both male and female assays to be carried out, the ether solution was simply washed with water and dissolved in the requisite volume of oil.

If, however, it was desired to separate the male and female activity, the ether solution was shaken with 10 separate portions of 10% aqueous sodium hydroxide. In this partition, the volume used was ether 75 cc. and alkali 50 cc. The alkali treatment removes about 95% of the estrogenic activity and no detectable amount of male hormone. The ether solution, after washing free from traces of alkali, was made to volume and assayed upon capons. The alkali extracts were combined and after acidification were shaken with 3 portions of ether, washed and made to volume for assay upon spayed female rats.

The next point was, how long must the extraction continue for quantitative values? After testing at various intervals, it was found that if the urine were extracted with 10 times its volume of fresh

benzene, no further activity, either male or female, could be extracted. (Table I.) The complete absence of male or female hormone in the re-extracted urine was demonstrated by the more rigorous technique of adding the second extract to the first in order to detect smaller amounts than might otherwise be assayed. This experiment and others similar to it convinced us that extracting the urine with 10 volumes of benzene in such an apparatus as we have used results in complete removal of both male and female hormones.

TABLE I.
Extraction of Sex Hormones from Acidified Male Urine by Benzene.

| Treatment | Sample | International units/liter Male hormone | Estrogenic |
|---|---|---|---|
| Boiled 2 hr.* | 4 | 25 | 80 |
| " 2 " | " | 21 | 85 |
| " 2 " | " | 18 | 100 |
| Not boiled | " | 22 | 25 |
| Boiled 2 hr. | 5 | 53 | 95 |
| " 2 " | " | 46 | 110 |
| " 2 " | " | 84 | 95 |
| Same urine re-extracted | | 0 | 0 |

*In this case the urine was introduced into the extraction by a pump delivering the urine at one-tenth the rate at which benzene was distilled.

We had known for some time that acid hydrolysis beyond making urine acid enough to extract without emulsification was not necessary to obtain maximum yields of male hormone. This is illustrated in Table I and is confirmed by our later works upon adsorption. However, since it was equally certain that the estrogenic activity was markedly increased by boiling with acid, it was necessary to determine whether the hydrolysis would destroy the male-hormone activity. The urine was acidified by adding 100 cc. of commercial hydrochloric acid per liter and then under reflux was boiled for 2 hours. This seemed the optimum time from the work of Cohen and Marrian and our preliminary results confirmed them. No difference could be found between 50 cc. of HCl and 100 cc. HCl per liter of urine. We preferred, however, to use the higher acid concentration since we did not autoclave the urine as did Cohen and Marrian. As can be seen from Table I, no loss of male hormone nor any increase is observed subsequent to acid hydrolysis. The effect upon the estrogenic substance, however, is quite marked and is equal to an increase of 300 to 400%.

If male hormone assays alone are desired, an alternative procedure may be used. We have found that the male hormone may be almost quantitatively adsorbed by the diatomaceous earth sold under the

name of Dicalite. The untreated earth called Superaid is the best adsorbent thus far studied. The adsorption may be carried out immediately after acidification or after boiling the urine by shaking the urine with Dicalite, using 100 gm. per liter of urine. The degree of acidity seems to make no difference in the adsorption but from an alkaline urine no adsorption occurs. The earth is filtered and may be kept at least a year without deterioration and probably for a longer time.

In order to release the activity we have extracted the earth 3 times with fresh portions of *hot* 95% ethanol using each time 1 liter for 500 gm. of original earth. The alcoholic washings are combined, distilled until all alcohol is removed, extracted with ether and the ether solution then shaken with sodium bicarbonate, washed, and made to volume, as is the case with the benzene extracts. This treatment fails to remove any appreciable estrogenic substance from the urine. Table II illustrates the results obtained from a typical batch of urine. The agreement between adsorption and extraction indicates the adequacy of either method.

TABLE II.

Comparative Yields of Male Sex Hormone by Adsorption and Extraction Methods.

| Treatment of Urine | Adsorbent | Internat. Units |
|---|---|---|
| Acidified by $H_2SO_4$ (not boiled) | Dicalite | 25 |
| Boiled with HCl | " | 20 |
| Routine quantitative extraction with benzene | | 23 |

Various other adsorbents have been studied and the results will be reported elsewhere. This report summarizes only the most advantageous process for routine use in the study of male-hormone excretion.

*Summary and Conclusions.* 1. A method for the extraction and assay of male and female hormones from normal urines has been described. 2. This procedure consists in 2-hour acid hydrolysis of the urine, extraction with ten times the volume of benzene using the extractor described, and separation into male and female fractions by alkali. 3. The assay is conducted with standards in parallel and results expressed in international units. 4. An alternative procedure for male hormone alone using an adsorption process is likewise described.

8405 P

# Preparation of the Specific Polysaccharide of Type I Pneumococcus.

MICHAEL HEIDELBERGER AND FORREST E. KENDALL.*

*From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.*

It was shown[1] that preparation of the specific polysaccharides of Types II and III pneumococcus in the cold and without the use of strong acid or alkali gave products forming much more viscous solutions than did the polysaccharides isolated by the older methods,[2] but apparently differing otherwise from the older preparations only in their ability to precipitate more antibody from homologous rabbit antisera. These differences were attributed to the greater chain length of the molecules of the new preparations.

The acetyl polysaccharide from Type I pneumococcus has now been similarly prepared. This product had the chemical and physical properties of the acetyl polysaccharide reported by Avery and Goebel[3] but gave solutions of much higher viscosity than did samples prepared according to Reference 3, and precipitated twice as much antibody nitrogen from a Type I antipneumococcus rabbit serum. Thus a solution of the new preparation, S 120, containing 1 mg. per ml. in 0.9% saline had a relative viscosity, $\eta_r$, of 1.69. After 8 hours in a sealed tube at 100° this solution showed $\eta_r = 1.05$. After treating with N/2 NaOH for 40 hours at 37° C. $\eta_r$ was 1.20. A preparation, S91a, isolated from culture filtrate concentrated on the steam bath gave $\eta_r = 1.10$.

Table I shows the amount of antibody nitrogen precipitated from rabbit and horse Type I antipneumococcus sera by an excess of the different preparations of Type I pneumococcus specific polysaccharide (SI).

As was found with S II and S III the use of heat as in the initial concentration of S I lowers its power to precipitate rabbit antisera without greatly affecting its reaction with horse antisera. Contrary to the S II and S III, treatment with alkali reduces the ability of S I

* The work reported in this communication was carried out under the Harkness Research Fund of the Presbyterian Hospital.

[1] Heidelberger, M., Kendall, F. E., and Scherp, H. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 188.

[2] Heidelberger, M., Goebel, W. F., and Avery, O. T., *J. Exp. Med.*, 1925, **42**, 727.

[3] Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1933, **58**, 731. Enders, J. F., and Pappenheimer, A. M., Jr., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 37.

TABLE I.
Antibody Nitrogen Precipitated by 0.20 mg. SI.

| Antiserum | SI preparation used: S120 | S120 heated | S120 alkali treated | S91a[3] | Deacetylated SI[2] |
|---|---|---|---|---|---|
| | mg. | mg. | mg. | mg. | mg. |
| Horse antibody B 77* | .76 | .72 | .66 | .71 | .63 |
| Rabbit serum 3.70* | .47 | .26 | .14 | .21 | .08† |

*Previously absorbed with "C" polysaccharide and Pneumococcus protein "R". N determinations by Dr. D. L. Shrivastava.
† N pptd. by 0.05 and 0.10 mg. S I.

to precipitate antisera from both species. Avery and Goebel[3] have shown this to be due to the removal of a labile acetyl group. The effect of heating appears to be related to the length of the chainlike molecules of the polysaccharide and is being studied further.

## 8406 C

### Evidence from Dwarf Mice Against the Individuality of Growth Hormone.

ROBERT W. BATES, THEOPHIL LAANES AND OSCAR RIDDLE.

*From the Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, N. Y.*

Evans and Long[1] were the first to obtain body growth from anterior pituitary extracts. Later investigators have uniformly considered the growth hormone a single entity, with the exception that recently Riddle and Bates—partly on the basis of data presented here—have sharply questioned this view.[2-5] On a wholly general view it seems to us that since hormones of the anterior lobe act upon or through other endocrine glands (thyroid, suprarenal, pancreas, parathyroid, gonads), most of which (gonads excepted) produce hormones highly important in healthy body maintenance, these latter glands probably take part also in normal body growth. If this is true the repair of the several disabilities caused by hypophysectomy

[1] Evans, H. M., and Long, J. A., *Anat. Rec.*, 1921, **21**, 61.
[2] Riddle, O., *Endocrinology*, 1935, **19**, 1.
[3] Bates, R. W., and Riddle, O., *J. Pharm. and Exp. Therap.*, 1935, **55**, 365.
[4] Riddle, O., and Bates, R. W., *Carnegie Year Book*, Dec., 1935.
[5] Riddle, O., Smith, G. C., Bates, R. W., Moran, C. S., and Lahr, E. L., *Endocrinology*, 1936, **20**, 1.

should be accompanied by marked growth; and, it is in fact in hypophysectomized animals that the most striking responses to "growth hormone" preparations have been obtained. Our study shows that gonad-stimulating (F.S.H.) preparations from pregnant mare's serum have no effect on body growth in the dwarf mouse. Prolactin and thyreotropic are well defined anterior lobe hormones which are here shown to be capable of stimulating growth in the dwarf mouse when given separately, and to exhibit synergism of the growth response when given together. The broad bearing of this evidence is attested by other data[5] which show that in their effects on the B.M.R. of normal and hypophysectomized pigeons these 2 hormones bear a precisely similar relation to each other.

The dwarf mice used (usually 38-150 days old) are from MacDowell's colony of the strain of hereditary dwarfism found by Smith and MacDowell[6, 7] to have pituitaries wholly deficient in eosinophiles, and to have deficient thyroids and adrenals which could be restored by daily implants of pituitaries of normal mice. Although A.P. implants from normal mice induced fairly rapid body growth in the dwarfs, the one test made with implants of dwarf pituitaries wholly failed to induce growth. Snell,[8] and MacDowell and Laanes[9] have shown that moderately good growth (considerable excess of fat) can be obtained from the feeding of desiccated thyroid to the dwarf mouse. Kemp[10] induced some growth in dwarf mice of this strain by injections of an extract ("growth hormone") which, from its method of preparation, must have contained all A.P. hormones.

All of the preparations used here were previously tested or assayed on immature male ring doves; that study either verified the absence or attested the presence of prolactin, F.S.H. and thyreotropic hormone in a particular quantity. For one type of controls a number of available preparations of "growth hormone" were used; these too were subjected to the assays described above, and though F.S.H. was not detectable in some (Collip) preparations we have found both prolactin and thyreotropic in all these "growth hormone" preparations. The prolactin preparations used here were first made to test free of F.S.H. and thyreotropic, and later most of them were heated (at pH 7.5-8.0, 98°C. for 1 hr. or 60°C. for 5

[6] Smith, P. E., and MacDowell, E. C., *Anat. Rec.*, 1930, **46**, 249.

[7] Smith, P. E., and MacDowell, E. C., *Anat. Rec.*, 1931, **50**, 85.

[8] Snell, G. D., *Anat. Rec.*, 1930, **47**, 316.

[9] MacDowell, E. C., and Laanes, T., *Carnegie Inst. of Wash., Year Book*, 1932, **31**, 47.

[10] Kemp, T., *Klin. Wochenschr.*, 1934, **13**, 1854.

hrs.) before use. This heating, at this pH, should further guard against traces of F.S.H. and thyreotropic; and if a "growth hormone" has the heat lability commonly assigned to it, that hormone should be destroyed by this procedure. All of the thyreotropic preparations used tested free (No. 267) or practically free (No. 243) of prolactin, but all contained F.S.H. which (as noted above) does not affect growth in the dwarf and also has no effect on the B.M.R. of pigeons; most of the thyreotropic preparations were heated (at pH 5.0) before use on the dwarfs.

TABLE I.
Growth Response of Dwarf Mice to Anterior Pituitary Preparations.

| Preparation or Hormone | Sex | Dose per day | Days injected | —Weight— Start | End | % Initial Wt. |
|---|---|---|---|---|---|---|
| Phyone 41B "Growth H" | ♂ | 0.1 cc. | 33 | 7.0 | 19.5 | 280 |
| " 41B " | ♀ | 0.1 " | 33 | 8.2 | 20.0 | 245 |
| " 41C " | ♂ | 0.06 " | 33 | 7.0 | 13.4 | 192 |
| " 44 " | ♂ | 0.1 " | 33 | 10.0 | 26.0 | 260 |
| Collip's Q21 " | ♂ | 0.1 " | 33 | 7.0 | 12.0 | 172 |
| " Q21 " | ♀ | 0.1 " | 33 | 7.0 | 9.5 | 136 |
| " Q16 " | ♂ | 0.1 " | 22 | 8.5 | 10.3 | 121 |
| " Q16 " | ♀ | 0.1 " | 22 | 7.0 | 9.5 | 136 |
| " POG " | ♂ | 0.2 " | 33 | 6.6 | 10.8 | 164 |
| " POG " | ♀ | 0.05 " | 33 | 8.6 | 11.5 | 134 |
| Lee-Schaeffer 720 " | ♂ | 0.15 " | 27 | 8.5 | 14.6 | 172 |
| " " " " | ♀ | 0.15 " | 27 | 7.8 | 12.0 | 154 |
| Thyreotropic 243* | ♂ | 0.8 mg. | 33 | 6.6 | 8.9 | 135 |
| " " | ♀ | 0.8 " | 33 | 7.2 | 9.6 | 133 |
| Prolactin 237* | ♂ | 0.8 " | 33 | 5.7 | 8.2 | 144 |
| " " | ♀ | 0.8 " | 33 | 6.0 | 7.1 | 118 |
| Pro. 237* + Thyr. 243* | ♀ | 1.6 " | 33 | 8.2 | 19.0 | 230 |
| (0.8 mg. of each) | ♀ | 1.6 " | 33 | 8.0 | 17.0 | 213 |
| Prolactin 380* | ♀ | 0.7 " | 33 | 9.4 | 12.0 | 128 |
| " " | ♀ | 5.0 " | 33 | 9.5 | 14.0 | 147 |
| Desiccated thyroid | ♂ | 2.0 " | 33 | 7.5 | 13.8 | 184 |
| " " | ♀ | 0.4 " | 33 | 8.0 | 12.2 | 153 |
| " " | ♀ | 0.4 " | 33 | 7.8 | 11.4 | 146 |
| Des. thyroid + 380* | ♀ | 1.1 "† | 33 | 6.4 | 10.4 | 163 |
| " " + 380* | ♀ | 0.5 "‡ | 33 | 7.3 | 10.6 | 146 |
| Prolan-Elberfeld | ♀ | 0.15 " | 20 | 6.7 | 6.2 | 93 |
| Mare serum | ♀ | 0.1 cc. | 10 | 7.2 | 5.3 | 74 |
| " " | ♀ | 0.1 " | 20 | 8.6 | 8.5 | 99 |

*Boiled or heated preparation.
†Used 0.7 mg. of 380, 0.4 mg. of thyroid.
‡ " 0.1 " " 380, 0.4 " " "

Table I records results of tests covering 33 days of injection which are not otherwise shown on Fig. 1. Certain of our results, such as alternate periods of injecting prolactin and thyreotropic, can not be satisfactorily shown in the table. Because the increases in weight are proportional to the initial weight of the mice we express growth in terms of percentage of initial weight, *i. e.*, 100 × final weight/initial weight (gm.).

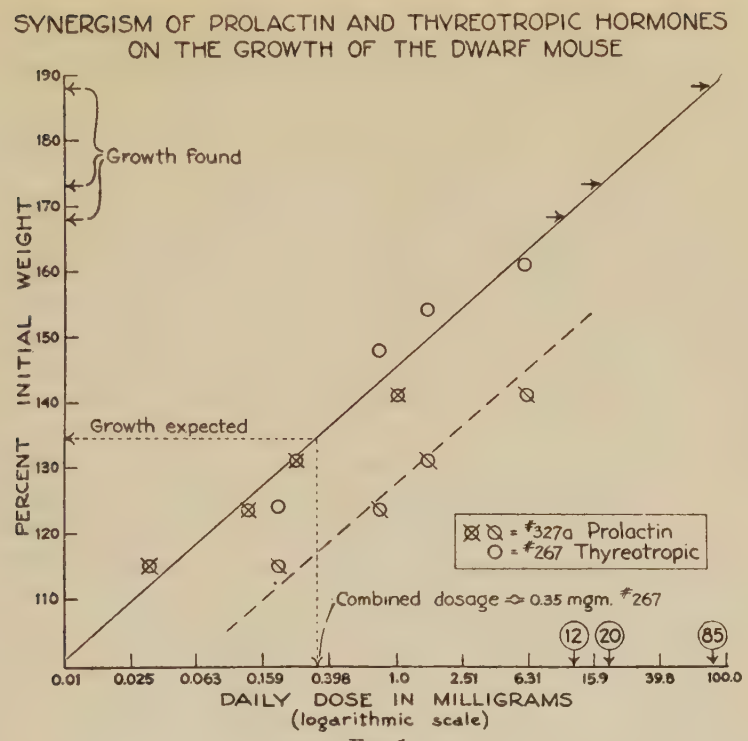



FIG. 1.

It will be noted that 3 different preparations of Phyone (growth hormone), in 0.1 cc. daily dosage, gave weights from 192-280% of the initial weights in 33 days. Either prolactin or thyreotropic causes growth, but a much slower growth. Are our preparations of the 2 latter hormones contaminated with the alleged growth hormone, or do they cause growth—directly or indirectly—by replacing different secretions of the deficient pituitary? In the former case one expects a combination of the 2 fractions to give additive effects upon growth; in the latter case an augmented effect, or synergism, is expected. This point was tested nearly 2 years ago by injecting 2 mice with 0.8 mg. daily of prolactin No. 237 (boiled 1 hr. at pH 7.5), 2 mice with 0.8 mg. daily of thyreotropic (+F.S.H.) No. 243 (boiled 1 hr. at pH 5.5), and lastly 2 mice with 0.8 mg. daily of each of the 2 preparations. The weights after 33 days in percentage of initial weights, for No. 237 were 144 and 118%; for No. 243 these were 135 and 133%; for the combination, 230 and 213%. One of us referred to the significance of this experiment 18 months ago, though no data were then given.[2]

In order to obtain a measure of the amount of synergism obtained by a combination of the 2 hormone preparations it is necessary to know the relationship between dosage and response (growth). Hence we have carried out the following preliminary experiment: Four dwarf mice 54-106 days old (wt. 6.8-8.6 gm.) were injected daily for 33 days with 0.2, 0.8, 1.6, and 6.4 mg., respectively, of thyreotropic (+F.S.H.) No. 267. Four other dwarf mice 54-83 days old (wt. 7.1-8.6 gm.) were injected daily with similar doses of prolactin No. 327a. When the growth obtained from the 8 mice is plotted against the logarithm of the dose (Fig. 1), the points fall along straight lines which have essentially the same slope. The mice receiving No. 327a (circles with diagonal line) did not grow as much per mg. of injected material as the mice on No. 267 (circles), hence the prolactin preparation is relatively less potent (about one-sixth). If the dosages with No. 327a are divided by 6 and the data again plotted (circles with crosses), one obtains 8 points from which to check the relation between dose and effect—the largest dose being 192 ($6 \times 32$) times greater than the smallest.

At the same time, 2 dwarf mice, 28 and 45 days old (wt. 6.5 and 7.7 gm.), received daily for 33 days a mixture of 0.3 mg. No. 267 (thyreotropic) and 0.3 mg. No. 327a (Prolactin). Since for promotion of growth 0.3 mg. of No. 327a is equivalent to about 0.05 mg. of No. 267, the combined dosage is equivalent to 0.35 mg. of No. 267. Assuming that a separate entity, growth hormone, contaminates both preparations the expected growth in the 2 mice is 135%. Instead, we obtained growths of 168 and 188%; this is an amount which would require dosages of 12 and 85 mg. respectively of No. 267 alone, and these quantities are 34 and 242 times the dosage actually given. One other dwarf (150 days old, wt. 7.5 gm.) received 0.8 mg. No. 327a + 0.2 mg. No. 267 daily for 33 days (equivalent to 0.33 mg. No. 267). The growth obtained was 174%; this is equivalent to 20 mg. of No. 267, or to 60 times the dosage actually given

*Summary.* Prolactin alone, desiccated thyroid alone, or thyreotropic hormone probably unaffected by any contaminating constituent of the preparations used by us, promotes growth in the dwarf mouse. In these mice prolactin and thyreotropic show a synergistic action (32 to 242×) upon body growth. Though the concept of a growth hormone as an individual entity has been useful it does not seem to be true.

8407 P

## Fibrinolysis of Hemolytic Streptococci and Their Variability.

RALPH R. MELLON AND FRANK B. COOPER.

*From the Institute of Pathology, The Western Pennsylvania Hospital, Pittsburgh.*

Our interest in the above correlation takes origin as follows: For the past 8 or 9 years one of us (Mellon) has studied with encouraging results a new serum approach to the therapeusis of hemolytic streptococcal infections. Treatment of the cases has been conducted exclusively in this hospital. Accompanying the serum's use we have frequently observed an *in vivo* dissociation of smooth (or smooth-mucoid) cultures into more saprophytic colony types—sometimes characterized by rough-colonied hemolytic streptococci, sometimes by typical non-hemolytic diphtheroids, wholly avirulent.

Of special significance is the fact that such dissociation seems to be a direct sequence of a clinical crisis on the part of the severe cases, whose ultimate recovery is thereafter no longer in doubt. Obviously, a serum approach that would transform virulent organisms into their non-virulent phases, is rather different from the conventional bactericidal or antitoxic ones.

Only an occasional observer (Hadfield, *et al.*[1]) in the field of fibrinolysis has directed attention to a correlation of virulence (as indicated by colony type) and fibrinolysis. Recently Tunnicliff[2] has shown correlation in a general way between R and S streptococci with respect to the increasing lysis and virulence as one proceeds from R to S. Reich[3] has shown that serial animal passage transformed a fibrinolytic strain of human origin into a non-fibrinolytic one suggesting a bovine origin; (Lancefield classification[4]), and which on serial subculture returned to its original fibrinolytic state. Accordingly, we have wondered if this correlation might extend to certain variants such as the above; more particularly to diphtheroids *whose in vivo dissociation from the streptococci had been confirmed by their occurrence in vitro, under controlled conditions.*

As an example of such a strain we wish to direct attention to a small-colonied hemolytic streptococcus from a treated case of cellulitis (Stoddard, small, No. 1—See Table). From a large-colonied hemolytic dissociant of this strain, typical non-hemolytic diphthe-

1 Hadfield, G., Magee, V., and Perry, C. B., *Lancet*, 1934, **1**, 834.

2 Tunnicliff, R., *Arch. Path., Soc. Trans.*, 1935, **20**, 811.

3 Reich, T., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 639.

4 Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

TABLE I.

The fibrinolytic activity of these cultures and of a strongly fibrinolytic control (Bloom, No. 9) was determined by the method of Tillett and Garner[5] against the plasma of a presumably normal individual. The figures in the horizontal columns represent the time of lysis of determinations run at different times. Figures in parenthesis show % of fibrinolysis at the end of 24 hours.

| Strain No. | Culture and Source | Time (Given in minutes, unless otherwise indicated) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | Stoddard, small colonies. Cellulitis | 90 | 35 | 40 | 60 | 105 | 60 | 50 |
| 2 | Stoddard, large colonies. Diphtheroid variant | | 0 | (10) | 0 | 0 | (10) | 0 |
| 3 | Stoddard, H. Large colonies. Variant | (90) | (90) | 13 hr. | (90) | (90) | <22 hr. | <22 hr. |
| 4 | Bovine III. Diphtheroid TBC variant | (50) | (50) | (+) | (50) | (25) | (50) | (50) |
| 5 | Sporoid. Avian TBC variant | (75) | (75) | (90) | (50) | (75) | (75) | (50) |
| 6 | H. Streptococcus (Miller). Sepsis | 75 | 90 | 120 | 75 | 75 | 105 | 105 |
| 7 | Streptococcus (Miller), non-hemolytic variant | 60 | 75 | 90 | 75 | 90 | 90 | 60 |
| 8 | Diphtheroid (Miller). Variant | (25) | (10) | (50) | (25) | (25) | (25) | (+) |
| 9 (Control) | H. Streptococcus (Bloom). Sepsis | 8 | 30 | 30 | 9 | 10 | 22 | 60 |

roid bacilli, producing a still larger colony have been derived. (Stoddard, No. 2). They in turn have reverted to the original hemolytic, small-colony type, as well as to an intermediate type; that is to say, to a hemolytic diphtheroid of large colony type (Stoddard H, No. 3). These dissociants are being studied by Philip Hadley, who will report on them in detail in a future communication. Reference to the table will show that Strain No. 3 is intermediate in its fibrinolytic activity, when compared to the actively lysing small (virulent) colony and the rather inactively lysing diphtheroid colony (non-virulent).

Similar results were obtained with the dissociants of another culture known as the "Miller" strain, which was isolated from the blood of a patient in pure culture in 1931. This hemolytic streptococcus (No. 6) was dissociated *in vitro* on 6 separate occasions into a non-hemolytic diphtheroid (No. 8). The latter was reverted to a streptococcus resembling the original, *but lacking hemolysis* (No. 7). Nevertheless, it was identical fibrinolytically with the hemolytic strain; and also serologically by the agglutinin-adsorption test, proving rather conclusively that the diphtheroid (No. 8) was not a contaminant. The latter did not cross-agglutinate and its lytic pow-

ers, although definite, are negligible in comparison with those of the streptococcus. Although the examples given in the table are but a fraction of the diphtheroid strains studied, the low degree of fibrinolysis is characteristic for many of them.

The diphtheroids with acid-fast granules, Bovine III (No. 4), and Avian sporoid (No. 5), are known to be in the tubercle cycle, by reason of the inter-transformability existing between them and the tubercle group. Yet they are indistinguishable in their fibrinolytic ability from diphtheroids dissociated from streptococci. A greening streptococcus (Hamilton strain, not in table), and a diphtheroid dissociating from it, both gave the same reaction as the Avian sporoid No. 5. These results are not strictly in accord with the work of Tillett and Garner,[5] *et al.*, who limited the phenomenon of fibrinolysis to hemolytic streptococci and an occasional staphylococcus.

## 8408 C

### Nonspecificity of the Chloride Impoverishing Mechanism of Small Intestine.

RAYMOND C. INGRAHAM. (Introduced by Maurice B. Visscher.)

*From the Department of Physiology, University of Illinois, College of Medicine, Chicago.*

Burns and Visscher[1] have shown that an isotonic solution of a mixture of sodium chloride and some other sodium salt with an indiffusible anion when placed in a loop of the small intestine becomes practically chloride-free in 1½ hours. This movement of sodium chloride, against a diffusion gradient into the blood through the intervention of the intestinal epithelium, resembles the removal of chloride by the kidney tubules from the glomerular filtrate. The intestinal preparation affords a convenient method of studying salt movement which may be of general importance.

As a step in elucidating this mechanism it was decided that the specificity of mechanism should be determined. Sodium bromide was chosen as an example of a salt similar chemically to sodium chloride but not a usual constituent of body fluid. It is the purpose of these experiments to see if, in the presence of an indiffusible

[5] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[1] Burns, H. S., and Visscher, M. B., *Am. J. Physiol.*, 1934, **110**, 490.

anion, sodium bromide will diffuse from a certain concentration in the intestinal fluid to the blood where a higher concentration exists.

Dogs were fed 10 gm. of sodium bromide per day for 3 days. At the end of this time about two-thirds of total plasma halides was bromide. Sodium amytal was used as an anesthetic. A loop of the lower portion of the ileum, sufficient in size to hold about 50 cc. of fluid, was tied off, cannulated and flushed with warm saline. The test solution, containing 40 mM/1 sodium chloride, 40 mM/1 sodium bromide and balance to isotonicity of sodium sulphate, was placed in the loop and allowed to remain 1½ hours. Samples were withdrawn at intervals for analysis of chloride and bromide in plasma and intestinal fluid by the electrometric technique of Hastings and Van Dyke.[2]

The results of a typical experiment are shown in Fig. 1. Four such experiments were performed with similar results. The original concentrations of both chloride and bromide in the intestinal fluid are lower than the corresponding concentrations in the plasma. In spite of these concentration gradients both chloride and bromide move from the intestine into the blood. After one-half hour the rates of removal are about the same. The ratios between the con-

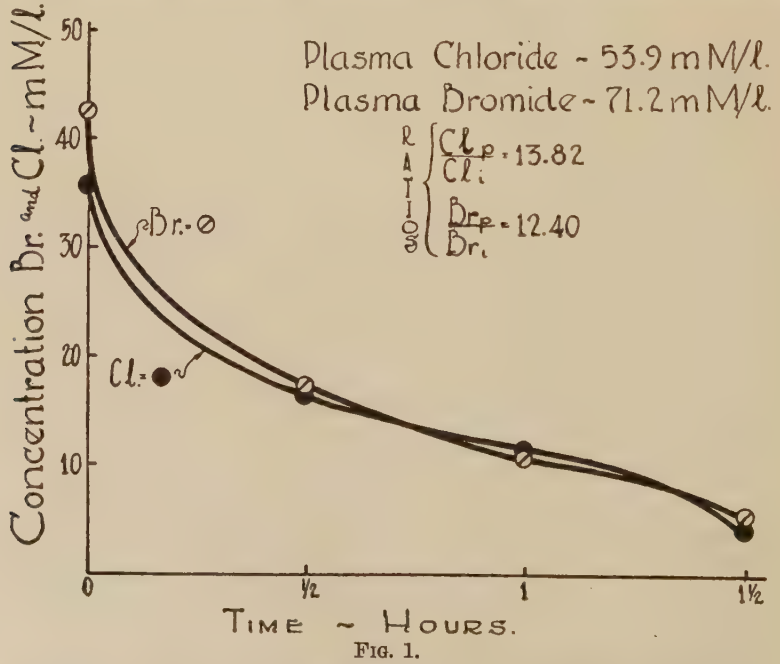



FIG. 1.

[2] Hastings, A. Baird, and Van Dyke, H. B., *J. Biol. Chem.*, 1931, **92**, 13.

centrations of the 2 ions in plasma and intestinal fluid are also approximately equal.

The rôle played by the intestinal epithelium, in this type of salt removal, is not specific for sodium chloride. This, together with the close agreement of the chloride and bromide concentration ratios, points to some physical process rather than a specific chemical one as the mechanism by which the movement is brought about.

8409 P

## The Mammary Gland in the Normal Adolescent Male.

FREDERIC T. JUNG AND ARTHUR L. SHAFTON.

*From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, and the Illinois Soldiers' and Sailors' Children School, Normal, Ill.*

The mammary gland of the human male, though generally dismissed as vestigial, deserves more attention than it has received. Like its counterpart in the female, it is subject to both inflammatory and neoplastic disease. It occasionally increases to such a size that the resulting gynecomastia becomes a medical or surgical problem, and actual lactation has been reported repeatedly.[1] Although the phrase "mastitis adolescentium" occurs in the literature,[2] we have not found any data on the frequency of this condition nor on the behavior of the gland in the normal pubescent boy. Unpublished observations on 49 boys in southern Wisconsin convinced Jung that a certain degree of mammary hypertrophy must be a regular feature of normal pubescence. The present study of a larger series was undertaken partly for the purpose of testing the correctness of this conviction, and was made possible by the generous cooperation of the Department of Public Welfare of the State of Illinois.

One purpose, then, was to obtain data analysis of which would give a chronology of the mammary changes as compared with the other phenomena of puberty. Hence the following observations were made: date of birth; weight; height; bi-iliac diameter; color of hair; amount* of hair on the pubes, in the axillae, on the face (*i. e.*, cheeks, upper lip, and chin), and on the chest; diameter of right areola; height of right nipple above surrounding skin; size* of

[1] Haenel, H., *Münch. Med. Wochensch.*, 1928, **75**, 261.

[2] Kriss, B., *Arch. f. Gynäkol.*, 1930, **141**, 507.

the subareolar node on the right; height of left nipple above surrounding skin; size* of the subareolar node on the left; pitch of voice; sizes of right and left testicle. The quantities marked * had to be, of necessity, graded in arbitrary units from 0 to ++++. The bi-iliac diameter was taken with an obstetrical caliper. The heights of the nipples were measured by using a micrometer screw mounted on 3 legs so as to work vertically; some obvious sources of error in this method must be borne in mind in interpreting the data. The diameter of the right areola was measured with a Vernier caliper. The testicular sizes represent the results of applying the Vernier caliper to the scrotum in such a way as to obtain the least of the 3 diameters of the testicle considered as an ellipsoid; here also certain sources of error must be borne in mind.

The subjects examined were all active, well-nourished boys in good physical and mental health and with excellent opportunities for outdoor work and play. Altogether 169 were examined; the number of boys in each age-group can be seen in the legend to Fig. 1.

Fig. 1 represents graphically the frequencies (in percent) with which palpable subareolar nodes were found in the various age-

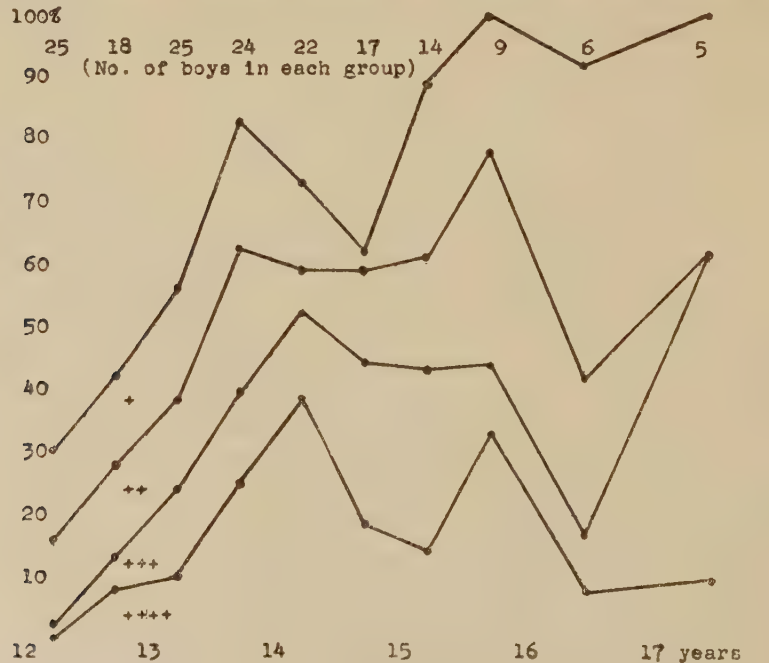



FIG. 1.

groups. From this it appears that the extreme (++++) enlargements of the node were most frequent in the 14.25 age group. In this group, however, it was still impossible to feel a node under 27% of the nipples examined; in 5 (23%) of the 22 boys of this group no node could be palpated under either nipple. After 14.25 years, extreme enlargement was found less frequently, while moderate degrees were more frequent; in the 15.75 and 17.5 age groups (9 and 5 boys respectively) nodes of small or moderate size were felt under both nipples in every case.

The nodes thus palpated varied from very firm, spherical, circumscribed nodes to masses measuring (in diameter) more than 1.5 cm. and so vaguely defined that two independent observers would make conflicting notes. None of the enlargements seen in this series would ordinarily be called gynecomastia, and in none of the cases was there any suggestion of a secretion.

The most marked enlargement was seen in a boy, aged 15 yrs. and 5 mos. He was strikingly muscular, the heaviest in his age group (140¼ lbs.; the median was 113¼ for the 15.25 group), slightly above the median in height (64 in.; median 63 in.), and second largest in bi-iliac diameter (28.7 cm.; median 25.8 cm.). He had the adult distribution of hair on the pubes and in the axillae, had begun to shave, and had the beginnings of hair on his chest. His testicular diameters (3.02 and 2.78 cm.) slightly exceeded the average values (2.37 and 2.50 cm. for right and left respectively) for the 15.25 age group.

Another boy, aged 14 yrs. 10 mos., had an especially marked bilateral enlargement, and said that the lumps had been actually painful for about 2 months. They were still tender at the time of examination, but there was no redness, warmth, or discharge to complete the picture of inflammation. This boy occupied a position near, and slightly above, the medians for the 14.75 age group with respect to weight, height, and bi-iliac measurement; he had a moderate amount of pubic hair, the beginnings of axillary and facial hair, and no chest hair; his voice no longer had the high pitch of the pre-pubescent voice, and seemed to be changing at the time. His testicular measurements were 2.39 and 2.38 cm., and thus lay close to the averages (2.27 and 2.41 cm. for right and left respectively) found by us for the 14.75 age group. These 2 cases are mentioned to show that the extremes of normal mammary enlargement are compatible with normal genital development.

Further analysis of the data is being carried out, but the following conclusions can now be stated: Enlargement of mammary tissue is

probably an invariable accompaniment of adolescent changes in boys. It was noted in 73% of boys in the 14.25 age group, in which the height of the nipple and the incidence of marked enlargement were maximal, and in 100% of boys in the 15.75 and 17.5 age groups, in which both the height of the nipple and the incidence of marked enlargement had declined. Older age-groups will have to be examined to observe the complete regression of the phenomenon.

## 8410 C

## A Note on the Analysis of Sympatheticomimetic Action.

MICHAEL G. MULINOS AND RAYMOND L. OSBORNE.

*From the Department of Pharmacology, College of Physicians and Surgeons, Columbia University.*

Langley[1] enunciated the broad rule that the pharmacological activity of suprarenal medullary extracts upon plain muscle simulates that of electrical excitation of the sympathetic nerves supplying each particular muscle. Dale[2] discovered that ergot depressed or paralyzed the motor sympathetic nerves, leaving the inhibitory effects relatively unaffected. After ergot, epinephrine no longer raises the blood pressure, but lowers it. Froelich and Loewi[3] made the further discovery that cocaine magnifies the effect of epinephrine on blood pressure.

These peculiarities of epinephrine after ergotamine or cocaine have been advanced as criteria of sympatheticomimicity,[6] despite the fact that the inhibitor activity elicited by epinephrine is but slightly affected by these drugs. In analyzing sympatheticomimicity Tainter[7] especially has emphasized the value of the ergot reversal and of the cocaine synergism. He used anesthetized animals, so that reflex and brain stimulants were not so easily separated from drugs acting peripherally. It is obvious that when a drug is injected into anesthetized animals, the resulting effects are more complex than would be the case in adrenalectomized animals with the central nervous system destroyed. Ergot reverses the motor effects of stimulation of the sympathetic nerves, and of injected epinephrine, while cocaine augments both of these. It is possible to elicit the effects

[1] Langley, *J. Physiol.*, 1901, **27**, 237.

[2] Dale, *J. Physiol.*, 1905, **32**, lvii.

[3] Froelich and Loewi, *Arch. f. exp. Path. u. Pharm.*, 1910, **62**, 159.

of sympathetic stimulation by drugs which stimulate the central nervous system directly or reflexly; and also by a nicotine-like action on the sympathetic ganglia. Furthermore, the blood pressure

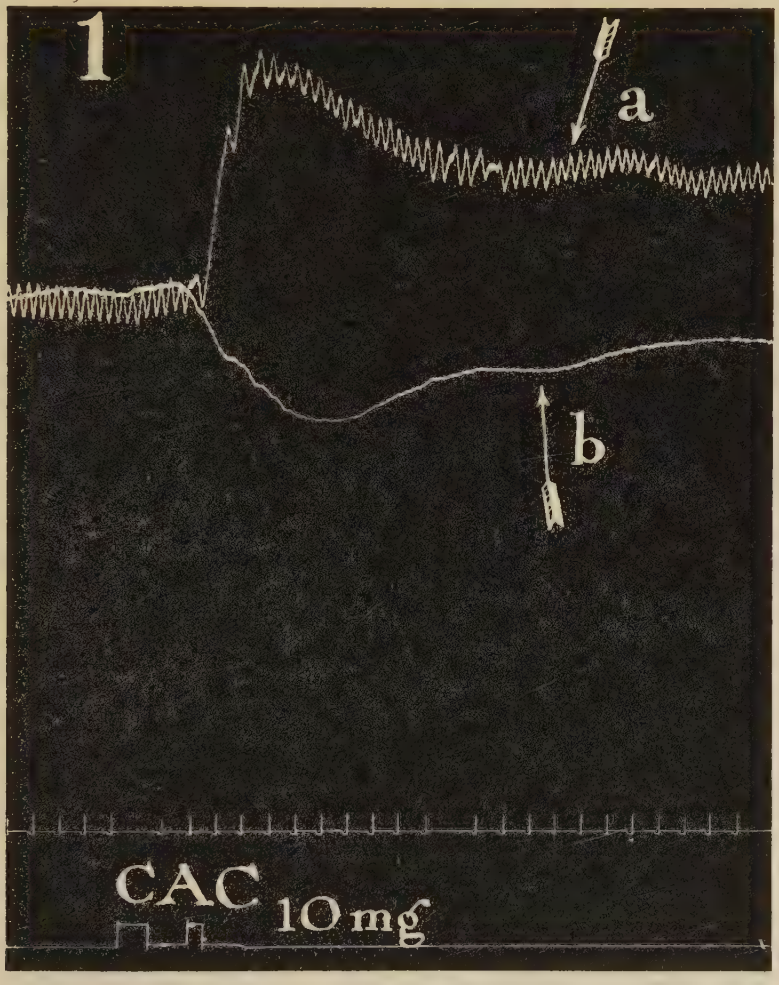



FIG. 1.

Blood pressure of cat anesthetized with Nembutal, 40 mg. per kilo. Atropine sulphate 5 mg. s. c. Tracing *a* was made before ergotamine. Tracing *b* was made after 1 mg. ergotamine tartrate. *CAC* is chloracetylcatechol. The "reversal" occurs also if the medulla is destroyed.

rise following stimulation of the central nervous system may be reversed by ergot and augmented by cocaine, as may that from electrical excitation of the splanchnic nerves.

Mulinos and Osborne[4] pointed out that chloracetocatechol (3,4-dihydroxyphenylchlormethylketone), designated for brevity as CAC, had properties suggestive of sympathetic stimulation. Ergot reverses the rise of blood pressure elicited by CAC on intact anesthetized cats (Fig. 1), while cocaine augments it (Fig. 2). From

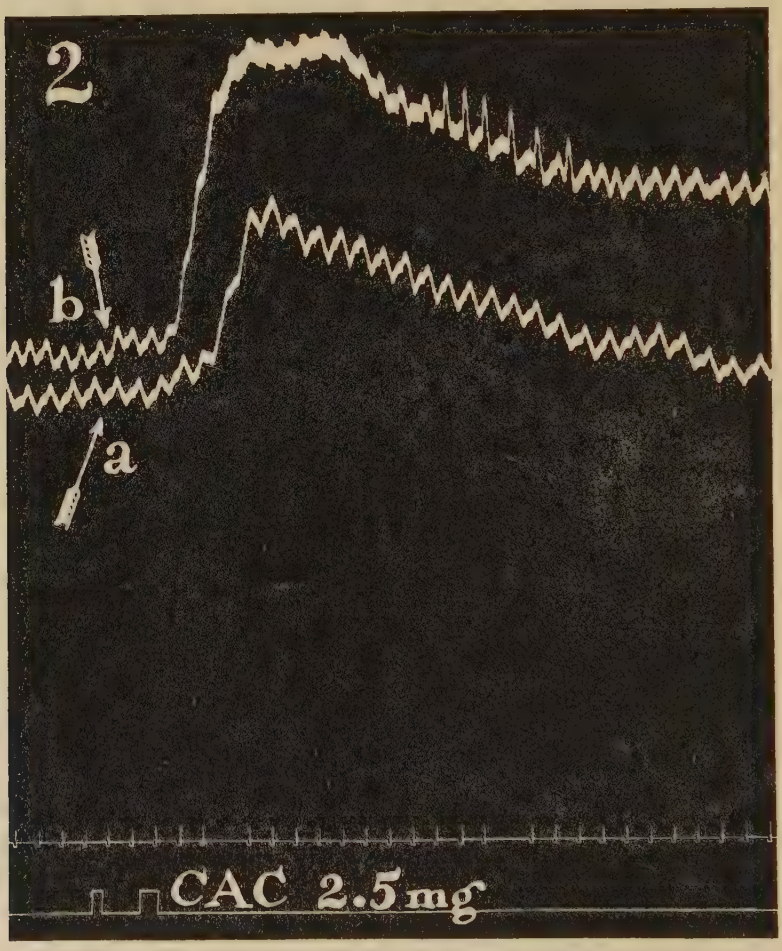



FIG. 2.
Cat blood pressure as for Fig. 1. Tracing *a* was made before cocaine. Tracing *b* was made after 5 mg. cocaine per kilo. The "synergy" does not occur if the medulla is destroyed. Compare with Fig. 1.

this it might be concluded that CAC resembles epinephrine in its action on blood pressure and in its behavior after ergotamine and

[4] Mulinos and Osborne, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1344.

cocaine. Table I shows clearly how erroneous this conclusion might be were it arrived at according to the criteria suggested by Tainter.[5-8]

TABLE I.
Summary of Blood Pressure Changes in Anesthetized Cats.

| Drug | Intact Cat | Pithed Cat | Ergotamine Cat | Cocaine Cat | Increase after cocaine |
|---|---|---|---|---|---|
| Chloracetocatechol | +140 | —27 | — 8 | + 94 | +37 |
| Catechol | + 41 | + 7 | + 4<br>— 1 | + 27 | To be reported |
| Epinephrine | +141 | +90 | —14 | +113 | +32 |
| Neosynephrine | + 45 | +70 | +10 | + 5 | |

The numbers refer to total injections in 31 cats. + means a rise. — means a fall. A blank means not tried.

Our experiments indicate that chloracetocatechol raises the blood pressure by stimulation of the medulla oblongata. Its action is "reversed" by ergot, and also by pithing; and "increased" by cocaine only if the medulla is intact. On the other hand, catechol acts to raise the blood pressure both in intact and in pithed animals, and its action is not reversed by ergot. Therefore we believe that experiments with arylamines and catechol derivatives, substances which stimulate also the medulla, must be performed on pithed animals in order that the criteria of ergotamine reversal and cocaine synergism may be applicable for the analysis of sympatheticomimetic activity.[6] When the precautions of pithing and of adrenalectomy are observed, an action on the sympathetic ganglia must be guarded against.

*Conclusion.* In the intact animal, ergot reversal and cocaine synergy of a blood pressure rise indicate that the impulses eliciting the rise reached the blood vessels by way of the sympathetic nerves; they do not indicate the site of origin of the impulses.

[5] Tainter, *J. Pharm. Exp. Therap.*, 1929, **36**, 569.
[6] Tainter, *J. Pharm. Exp. Therap.*, 1930, **40**, 43.
[7] Tainter, *J. Pharm. Exp. Therap.*, 1932, **46**, 27.
[8] Wirt and Tainter, *J. Pharm. Exp. Therap.*, 1931, **44**, 299.

8411 C

# Clinical Studies with the Thyreotropic Pituitary Hormone.

PAUL STARR.

*From the Thyroid Clinic, Northwestern University Medical School.*

Schittenhelm and Eisler[1] reported the effect of a thyreotropic pituitary hormone in human beings in June, 1932. The metabolic rate of a healthy young woman rose 20%. Eitel and Loeser[2] studied the effect of a Schering-Kahlbaum thyreotropic preparation. From 200 to 300 guinea pig units per day in patients gave uncertain results, but a dosage of 600 units a day caused a rise from +15 to +42% in 7 days. Ten patients were referred to. The dosage values are to be compared with those indicated later. Schittenhelm and Eisler[3] later reported that a man with a postthyroidectomy myxedema sustained no rise in basal metabolic rate with 10 daily injections of 600 guinea pig units of thyreotropic hormone, but during this time the blood iodine concentration rose from 6 to 16 gamma %. In a puerperal obese patient, 4 series of injections led to no increase of metabolic rate but a loss of 10 pounds in weight occurred in a month. Müller[4] gave from 200 to 600 guinea pig units of the Schering-Kahlbaum preparation daily to several patients in the later months of pregnancy, with no observable effects. A thorough study of acute effects of Schering-Kahlbaum and of I. G. Farben industrie thyreotropic preparations was reported by Feuling.[5] Twenty-eight patients receiving 4 daily injections of Schering-Kahlbaum thyreotropic preparation sustained an average rise in basal metabolic rate of 15%; 29 patients receiving the I. G. preparation sustained an average rise of 19%. No prolonged experiments were conducted. Wachstein[6] reported 2 significant cases. A patient with myxedema was very susceptible to the first series of thyreotropic injections, moderately reactive to the second series a month later, but unresponsive to the third series of large doses given in the third month. This may be the first report of refractoriness to thyreotropic medication in a human being. A case of hypophyseal cachexia did not respond to the same medication. Thompson,[7]

[1] Schittenhelm and Eisler, *Klin. Wchns.*, 1932, **11**, 1092.

[2] Eitel and Loeser, *Klin. Wchns.*, 1932, **11**, 1748.

[3] Schittenhelm and Eisler, *Klin. Wchns.*, 1932, **11**, 1783.

[4] Müller, H. P., *Klin. Wchns.*, 1933, **12**, 1899.

[5] Feuling, M., *Deutsches Arch., f. Klin. Med.*, 1933, **176**, 90.

[6] Wachstein, M., *Klin. Wchns.*, 1934, **13**, 1434.

[7] Thompson, W., *J. Am. Med. Assn.*, 1935, **104**, 252.

using Squibb's growth hormone preparation and Wilson's Phyone found a rise of basal metabolic rate in 24 of 39 patients. He pointed out that the complete myxedema patients failed to respond, while the symptoms and metabolic rates of patients with hyperthyroidism were increased. Lederer[8] reported 2 cases of Simmonds' disease treated with a thyreotropic preparation, preglandol, Roche; the metabolic rate rose during treatment but from 3 weeks to a month after treatment fell to even lower levels than had been present originally.

The thyreotropic pituitary hormone preparation used in this study is prepared by Dr. O. Kamm of Parke, Davis and Company. It is described as an aqueous solution of bovine anterior pituitary gland, substantially free of growth, and gonadotropic hormones representing the thyreotropic principle in purified form. It is supplied for experimental purposes under the name Antuitrin T. It contains less than 1% total solids, has a pH close to 7, and is non-irritating. The potency is about 50 guinea pig units per cubic centimeter. Twenty-four patients have been treated with this preparation.

In 4 normal adults one, receiving 3 injections of 1 cc. sustained a rise of basal metabolic rate from 0 to +22 on the eighth day; a second sustained an exactly similar rise but received 8 injections and 21 days were required for the highest elevation of metabolism to be reached; the third and fourth, receiving 5 and 6 injections respectively, increased their metabolic rates 20% in 5 and 7 days. There is thus great individual variation of response in normal individuals.

In 4 castrate women, one receiving 7 injections had a rise of basal metabolic rate of only 3% in 21 days; a second, receiving 8 injections had a rise from —15 to +8% in 21 days; the metabolic rate of the third and fourth cases rose 24% with 4 and 7 injections respectively in 5 and 7 days. There is, thus, great individual variation to this material among castrate women and their responsiveness seems the same as that of normal persons.

Six patients with hypothyroidism, simple and complicated, have been treated with from 6 to 8 daily injections of 1 cc. The basal metabolic rate of one patient with pituitary deficiency of traumatic origin, partial Simmonds' disease, rose from —30 to —11%; 2 patients with congenital hypothyroidism and one with complete adult myxedema failed to respond with any elevation of rate; one mild myxedema and one adolescent hypothyroidism responded to the same degree as the normal adults.

[8] Lederer, J. A., *Rev. Belge des Sciences Med.*, 1935, **7**, 369.

Four adult female patients with large goiters have been treated with 6 daily injections. In one the basal metabolic rate rose from +10 to +34%, with no determinable change in the size of the goiter. In one, no change in metabolic rate was observed but there was a rapid diminution in size of the goiter. An acute variation of metabolic rate may have been missed. In 2 others no distinct changes were observed.

Six patients with hyperthyroidism have been treated. One received only 6 daily injections, sustaining a rise of metabolic rate from +32 to +58. One received 15 injections in 2 months; one received 35 injections in 4 months; one received 112 injections in 11 months. Of these 3 patients, none indicated the development of refraction to the medication or development of thyroid inhibition. Lugol's solution was given during some of this time. One patient, receiving 21 injections, divided into 6 series, during 3 months, failed to increase her metabolic rate with the injections, and after the last series has shown a fall of metabolic rate to normal. One patient received 63 injections during 4 months. During this time Lugol's solution has been administered. The metabolic rate fell to normal in 6 weeks and remains so under Lugol's solution. In 2 of these patients great increase in metabolic rate and symptoms was induced by the thyreotropic hormone; iodine obliterated this effect in the other patients.

*Summary.* A review of the published reports of the clinical application of the thyreotropic pituitary hormone is given. Our experience with 24 patients is outlined. In normal patients there is great individual variation in reactiveness to the material, as indicated by the basal metabolic rate. This is also true in castrate women. Some patients with low metabolic rates respond, whereas others do not. Patients with goiters respond with similar individual variation. In one, a diminution of the goiter was observed. Acute temporary exacerbation of hyperthyroidism has been produced in 2 patients; this has been prevented by iodine in others; in one patient with hyperthyroidism refractoriness to the medication seemed present before treatment, and treatment was followed by a fall in metabolic rate.

8412 C

# Effect of Castration on Thyroid in Female Guinea Pigs.

PAUL STARR AND R. C. BRUNER.

*From the Departments of Medicine and Experimental Surgery, Northwestern University Medical School.*

A study of the literature on the effect of castration on the thyroid gland has shown many contradictory results. Earlier work seems to indicate a reduced thyroid activity in castrate animals. A decreased metabolic rate after castration has been reported by Loewy and Richter[1] and by Savage, Sherwood and Hall.[2] Histological studies by Korenchevsky,[3] Constantini[4] and Chouke[5] show a decreased activity of the gland and a decrease in the number of mitotic figures. A decrease in the weight of the thyroid and an atrophy of the gland have been reported by Anderson and Kennedy[6] and Bokelman.[7] Others have reported no marked change in the structure or weight of the gland, namely Leonard, Myer and Hisaw[8] and Freudenberger and Billeter.[9] More recent work by Bialet-Laprida[10] and Korenchevsky and Dennison[11] report a slight hypertrophy of the thyroid. Bates, Riddle and Lahr[12] in a quantitative assay of the occurrence of thyreotropic hormone in 7 types of cattle classified as to age, sex and stage of reproductive cycle, found a decreased amount of hormone in the pituitary gland of steers (adult, male castrates).

Contradictory results have been reported independently by Loeser and Aron. Loeser,[13] who has studied the thyreotropic hormone extensively, claims that 18 days after castration the thyroid gland of female guinea pigs is markedly stimulated. Other work by Loeser[14] indicates that the pituitary gland of a castrated animal when implanted into a normal animal will cause an increased activity of the

1 Loewy, A., and Richter, P. F., *Zentralbl. f. Physiol.*, 1902, **16**, 449.
2 Savage, Sherwood and Hall, *Am. J. Physiol.*, 1933, **105**, 741.
3 Korenchevsky, *Brit. J. Exp. Path.*, 1925, **6**, 21.
4 Constantini, P., *Rassegna internaz. clin. terap.*, 1929, **10**, 718.
5 Chouke, K. S., *Endocrinology*, 1930, **14**, 12.
6 Anderson, D. H., and Kennedy, H. S., *J. Physiol.*, 1933, **19**, 1.
7 Bokelman, *Arch. f. Gynak.*, 1931, **144**, 272.
8 Leonard, Meyer and Hisaw, *Endocrinology*, 1931, **15**, 17.
9 Freudenberger, C. B., and Billeter, O. A., *Endocrinology*, 1935, **19**, 347.
10 Bralet and Laprida, *Rev. Soc. Argent de Biol.*, 1933, **9**, 245.
11 Korenchevsky and Dennison, M., *J. Path. and Bact.*, 1934, **38**, 231.
12 Bates, R. W., Riddle, O., and Lahr, E. L., *Am. J. Physiol.*, 1935, **113**, 259.
13 Loeser, A., *Klin. Wschr.*, 1935, **14**, 4.
14 Loeser, A., *Klin. Wschr.*, 1934, **13**, 766.

thyroid gland, greater than the stimulation resulting from the implantation of the pituitary of a normal non-castrated animal. Aron[15] claims that castration increases the titrable amount of thyreotropic hormone in the blood.

I. The histology of the thyroid gland of female guinea pigs, 18 to 23 days after castration was determined by a study on 37 animals in 4 groups, with weights varying from 120 to 1030 gm. A bilateral ovariectomy was performed. Eighteen to 23 days later a unilateral thyroidectomy was done. In both operations ether anesthesia was used. No iodine was given during the operations or during the experiment. The interval of 18 to 23 days was found to be the period of maximum stimulation of the thyroid after castration by Loeser,[13] and consequently was used in this study. In general, those workers finding stimulated thyroid glands used guinea pigs weighing from 150 to 200 gm. while those reporting no stimulation used animals of greater weight and age; hence we used 4 weight groups. Group A, from 120-175 gm.; Group B, from 240-285 gm.; Group C, from 440-535 gm., and Group D, from 685-1030 gm.

Our conclusion, based on this histological study, is that there is no gross indication of increased thyroid activity after castration and in all cases the thyroid gland was found to be in a resting state with low, flattened epithelium and acini filled with colloid.

II. Using the thyroids removed by unilateral thyroidectomy in the previous study as control glands indicating a non-stimulated resting state of the remaining thyroid, and convinced by control studies 9 to 11 days after unilateral thyroidectomy, and by the work of Loeb,[16] that no compensatory hypertrophy had occurred, these castrate guinea pigs were injected with thyreotropic hormone (Antuitrin T, Parke, Davis and Company). The animals were injected with 0.025 cc. per 100 gm. of body weight (approximately 5 G.P. units per 100 gm.) daily for 4 days and were autopsied on the fifth day.

The histological findings were that all of the 37 animals responded to thyreotropic hormone with a degree of stimulation inversely proportional to the weight of the animal; that is, the smaller animals responded with maximum stimulation, while the larger animals responded to a lesser degree. Control non-castrates which had been unilaterally thyroidectomized (Group E) responded to thyreotropic hormone to a comparable degree. Control castrates in which a uni-

[15] Aron, M., and Benoit, J., *C. R. Soc. de Biol.*, 1931, **108**, 784.

[16] Loeb, L., *J. Med. Res.*, 1919, **40**, 199.

TABLE I.

| Group | No. of animals | Weight mean | Castrated | Days between castration and unilateral thyroidectomy | Histology removed thyroid | Days between unilat. thyroidectomy and injections | Medication | | | Histology thyroids removed at autopsy |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Prep. | Amt. Dose | No. Doses | |
| A | 10 | 140 | Yes | 22 | Normal. No stim. | 9-11 | Ant. T. | 0.025 per 100 gm. | 4 | Stim.++++ |
| B | 12 | 265 | ” | 18-20 | ” ” | 9-11 | ” | ” ” | 4 | ” ++++ |
| C | 9 | 480 | ” | 21 | ” ” | 9-11 | ” | ” ” | 4 | ” +++ |
| D | 6 | 875 | ” | 23 | ” ” | 9-11 | ” | ” ” | 4 | ” ++ |
| E Control | 2 | 265 | No | — | ” ” | 9 | ” | ” ” | 4 | ” ++++ |
| F Control | 2 | 270 | Yes | 21 | ” ” | 9 | — | — — | — | Normal. No stim. |
| G Control | 4 | 305 | ” | — | — | 30 | ” | ” ” | 4 | Stim.++++ |

30 days between castration and injections.

lateral thyroidectomy was performed but which were not given thyreotropic hormone, showed a thyroid gland that was not stimulated (Group F). Control castrates (Group G) in which no unilateral thyroidectomy was performed, responded to injections of thyreotropic hormone comparable to both normal animals and unilaterally thyroidectomized castrates.

In Table I we have designated the degree of stimulation of the thyroid gland as one to 4 plus. The first grade of hyperplasia was represented by a change to cuboidal type of epithelium in the central part of the gland; the second grade was indicated by this condition throughout the gland; the third grade by an increased height of epithelium throughout the gland with some loss in colloid; the fourth (++++) by universal maximum epithelial height, loss of the circular shape of the cut alveoli, and the absence of colloid as reported by us previously.[17]

III. Using basal metabolism as an indication of response to thyreotropic hormone, 23 castrate female guinea pigs were injected with hormone doses varying from 0.025 per day to 0.025 per 100 gm. body weight per day. Twenty of these animals responded with an increased metabolic rate varying from +18 to +60 after 3 to 5 injections. Three animals gave no histologic or metabolic response. This response is comparable to 37 normal non-castrate female guinea pigs responding to thyreotropic hormone injections on the third to fifth day with an increased metabolism of from +20 to +65.

In summary, we found the following: (1) No gross histologic change was found in the thyroid of untreated female guinea pigs 18 to 23 days after castration; (2) castrate female guinea pigs responded to thyreotropic hormone 30 days after castration with histologic changes in the thyroid no different from those found in normal female guinea pigs given the same treatment; (3) the metabolic response of castrate female guinea pigs to thyreotropic hormone is of the same magnitude as is the response of non-castrated female guinea pigs.

[17] Starr, P., Patton, H., and Bruner, R. C., Christian Birthday Volume, in press.

8413 C

## Effect of Acids on Certain Carbocyclic Antiseptics.

F. W. HARTMAN AND VICTOR SCHELLING.

*From the Department of Pathology, Henry Ford Hospital, Detroit.*

During the course of recent experimental studies on the pathology and treatment of burns directed toward reducing the incidence of infection associated with the Davidson tannic acid treatment, the addition of various antiseptics was considered. Aldrich[1] had pointed out the low antiseptic value of tannic acid alone and noted the frequency of infection beneath the heavy eschar. In view of his findings, Aldrich substituted crystal violet for tannic acid in the treatment of burns. Experimentally, burns treated with crystal violet, either alone or in combination with tannic acid, allowed the development of *B. pyocyaneus* and the colon group. Obviously, the proper antiseptic must be compatible with tannic acid, must have high bactericidal value against both cocci and bacilli, and low toxicity for tissue cells both locally and generally.

The carbocyclic group of compounds seemed to give the most promise of satisfying these criteria, and amyl tricresol (Upjohn and Company) was the first one tried. This material used in 1-1000 concentration with 5% or 10% tannic acid reduced the incidence of infection in experimental burns satisfactorily and at the same time did not interfere with the healing of the wounds.

Bactericidal tests (Food and Drug Administration Method) showed amyl tricresol effective in dilutions of 1-2000 against *E. typhi* and in dilutions of 1-4000 against *S. aureus* in 10 minutes. In combination with 5% tannic acid it became effective in dilution of 1-12,000 against *E. typhi* and 1-8000 against *S. aureus* in 10 minutes. Thus the effectiveness of amyl tricresol was increased 6 times against *E. typhi* and 2 times against *S. aureus* through combination with tannic acid.

Other organic acids have been found to have a comparable synergistic effect, *i. e.*, citric, tartaric, salicylic, benzoic, gallic, and lactic, when the pH of the solutions is maintained at a point between 2 and 3. Effectiveness is increased with greater concentrations of acid and decreased with dilution or buffering of the solutions.

Of the inorganic acids, only boric and hydrochloric were tested, and of these hydrochloric alone showed appreciable effectiveness.

[1] Aldrich, R. H., *New Eng. Med. J.*, 1933, **208**, 299.

Used in concentration of 0.36% by weight, this acid, combined with amyl tricresol, showed the effective dilution to be 1-28,000 against *E. typhi* and 1-24,000 against *S. aureus* in 10 minutes, increases of 17 and 6 times respectively over that of the tricresol. The striking increase in bactericidal activity due to the combination of tricresol with tannic and hydrochloric acids demonstrated by these observations placed these combinations in the group of most efficient antiseptics and led us to test other combinations as shown in Table I.

TABLE I.

| Substance (F. & D. A. Method) | Dilution effective in 10 minutes *E. typhi* 20° C. | *S. aureus* 37° C. |
|---|---|---|
| Amyl tricresol 1-2000 (U) Glycerine 50%, alcohol 25% | 2000 | 4000 |
| Amyl tricresol 1-2000 (U) Glycerine 50%, alcohol 25%, tannic acid 5% | 12000 | 8000 |
| Amyl tricresol 1-2000 (U) Glycerine 50%, alcohol 25%, HCl 0.36% | 28000 | 24000 |
| Dihydroxy-n-hexyl benzene (S) | 4000 | 4000 |
| " " , tannic 5% | 16000 | 4000 |
| " " , HCl 0.36 % | 28000 | 20000 |
| Di-hydroxy-di sec. hexyl-benzene (P) Glycerine 50%, alcohol 25% | 2000 | 52000 |
| Di-hydroxy-di sec. hexyl-benzene (P) Tannic acid 5%, glycerine 50%, alcohol 25% | 4000 | 52000 |
| Di-hydroxy-di sec. hexyl-benzene (P) HCl 0.36%, glycerine 50%, alcohol 25% | 20000 | 76000 |
| Di-hydroxy-hexyl-ethyl benzene 1-1000 (H) | 1000 | 24000 |
| Di-hydroxy-hexyl-ethyl benzene 1-2000 (H) HCl 0.36% | 32000 | 60000 |
| Di-hydroxy-hexyl-ethyl benzene 1-2000 (H) Tannic 5% | 20000 | 36000 |
| Di-hydroxy-hexyl-chloro-benzene 1-200 (H) Glycerine 50%, alcohol 25% | 2000 | 28000 |
| Di-hydroxy-hexyl-chlorobenzene 1-2000 (H) Glycerine 5%, alcohol 25%, tannic 5% | 24000 | 36000 |
| Di-hydroxy-hexyl-chloro-benzene 1-2000 (H) Glycerine 5%, alcohol 25%, HCl 0.36% | 48000 | 68000 |
| Chloro-thymol 1-2000 Glycerine 50%, alcohol 25% | 2000 | 2000 |
| Chloro-thymol 1-2000 Tannic acid 5%, glycerine 50%, alcohol 25% | 16000 | 12000 |
| Chloro-thymol 1-2000 HCl 0.36%, glycerine 50%, alcohol 25% | 32000 | 24000 |
| Di-hydroxy-di-octyl-benzene 1-1000 (P) Glycerine 20%, HCl 0.36% | 20000 | 28000 |
| Di-hydroxy-di-heptyl-benzene 1-1000 (P) Glycerine 20%, HCl 0.36% | 24000 | 28000 |
| Di-hydroxy-di-hexyl-chloro-benzene 1-2000 (H) HCl 0.36%, alcohol 90% | 35000 | 70000 |
| Hexyl-p-chloro-m cresol 1-2000 (H) Glycerine 20%, HCl 0.36% | 36000 | 200000 |
| Hexyl-p-chloro-m cresol 1-2000 (H) Tannic acid 5%, glycerine 20% | 4000 | 124000 |

U—Upjohn and Company. S—Sharp and Dohme. P—Parke, Davis Company. H—Henry Ford Hospital.

It is apparent from the data presented that many of carbocyclic compounds which have been considered relatively ineffective antiseptics or which have been effective against one group of organisms and ineffective against other groups are greatly enhanced in value in the first instance and become more uniformly effective against all groups of bacteria in the second instance by the combination with certain acids. The pH needs to be decreased only to 2 or 3 to get a marked increase in the effectiveness, and such relatively low pH is preferred because this adds little to the tissue toxicity. However, higher concentrations of acid will further increase the effectiveness and lower concentrations will decrease the effectiveness as will buffering the solutions. Aside from the pH there is a difference in acids as far as the effectiveness of the combination is concerned. For example, the pH of 5% tannic acid and 0.36% hydrochloric acid are comparable, but the combinations with the latter are invariably much more effective.

Although phenol coefficients are not calculated in this series, approximate phenol coefficients may be estimated by dividing the figure listed by 100. Further, it should be kept in mind that phenol coefficients calculated on the F.D.A. method range from 25 to 40% below those calculated on the Hygienic Laboratory method, since 0.5 cc. of culture is used in the former rather than 0.1 cc. as in the latter, and the specified strains of *E. typhi* and *S. aureus* are more resistant.

In addition to the surprisingly high effectiveness against bacteria of the combinations listed, experimental and clinical application to burned areas, granulating surfaces and wounds generally demonstrates that as a group they have low toxicity locally and no systemic evidence of toxicity has been found. Intravenous administration of the various dihydroxy benzenes (resorcinols) with hydrochloric acid 0.36% by weight is well borne by animals, and the effects on various bacteria in the circulating blood and upon the various organs will be reported in a later communication.

8414 P

# Induction of Polarity in Fucus Furcatus by a Localized Concentration of Hydrogen Ions.*

D. M. WHITAKER.

*From the School of Biological Sciences, Stanford University.*

The *Fucus* egg is spherical (diam. = 65-90μ). About 17 hours after fertilization (15°C.) a protuberance develops at one side of the egg (involving softening and extension of the cellulose wall, and ordinarily protruding in a plane parallel to the substrate). This protuberance extends (Fig. 1), and the plane of the first cell division passes across just back of the base of the protuberance, perpendicular to the direction of protrusion. This results in 2 cells of different shape and very different developmental fate or potentiality. The protuberance cell gives rise to the rhizoid of the new organism, the other cell to the thallus. When the point of development of the rhizoid protuberance is determined, the polarity and the developmental pattern of the whole embryo is determined.

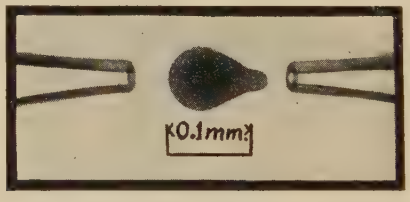



FIG. 1.
Egg developing in pH gradient. Medium is sea water at pH 7.8. Left pipette contains sea water agar (pH 7.8). Right pipette contains buffered acidified sea water agar (pH 6.4). Rhizoid protuberance develops at region of low pH.

Among conditions which affect the point of origin of the rhizoid is the presence of neighboring eggs which lie within a few egg diameters (sea water between). The rhizoid is induced to form on the side toward a neighbor, or in the resultant direction of neighbors.[1,2,3] One of the conditions which may be expected to obtain during this action at a distance is a gradient of hydrogen ion concentration, due to diffusion of $CO_2$ or other acid metabolites from each cell, with highest concentration toward the neighbor.

* Supported in part by funds granted by the Rockefeller Foundation. With technical assistance from Mr. Edward Lowrance.

[1] Kniep, Hans, *Jahrb. f. wiss. Bot.*, 1907, **44**, 635.

[2] Hurd, A. M., *Bot. Gaz.*, 1920, **70**, 25.

[3] Whitaker, D. M., *Biol. Bull.*, 1931, **61**, 294.

The experiments reported here are part of an investigation of the factors involved in the mutual influences and will be published elsewhere at greater length with additional data.

Gradients of hydrogen ion concentration were established across 50 individual eggs by means of micro-diffusion pipettes. Pipettes were drawn from 10 mm. pyrex tubing tapering to internal diameters of about 10-20 microns in a length of 1-2 cm. Tips were cut off squarely with diamond points, and pipettes were matched in pairs.

One pipette was filled from the tip with 1% agar sea water (pH range 7.4-8.3), the other with 1% agar and sea water buffered variously at pH 5.8 to 6.4. The pH was measured with a glass electrode. Buffer strengths were adjusted so that pH held constant within 0.1 unit for 24 hours with eggs developing in the mixture but there was little more than enough capacity to attain this effect. Thus upon being diluted in diffusing from the tip of the pipette the buffered mixture rapidly lost its buffer capacity and was therefore unable seriously to drop the higher pH on the other side of the egg.

Eggs were obtained and reared in a dark constant temperature room (15°C.), being exposed briefly only to red light. Soon after fertilization an egg was placed alone in a dish of sea water on the stage of a microscope on a Taylor micromanipulator. The 2 pipettes were mounted in the manipulator and the tips approached the egg on opposite sides, as in Fig. 1. The level of the contents of the pipettes was several millimeters above the level of the sea water in the dish to augment diffusion with a slight pressure flow and to increase the reserve of solutions. Results were recorded photographically.

Of 50 eggs, 46 (92%) developed rhizoids on the side of the egg toward the acid pipette. Of these, 24, or more than half, developed the rhizoid within 10° of a line toward the source of hydrogen ions. A typical good case is shown in Fig. 1. The same results were obtained using McIlvaines, or HCl-bicarbonate buffer.

*Summary.* Hydrogen ions in suitable concentration, when applied to a localized region of the *Fucus* egg in sea water, induce formation of the rhizoid protuberance. The polarity and the developmental pattern of the whole embryo are determined.

8415 P

# Excretion of Urea and Creatinine in the Dog in Relation to Rate of Urine Formation.

JAMES A. SHANNON.

*From the Department of Physiology, New York University College of Medicine.*

Austin, Stillman and Van Slyke[1] on the basis of data obtained on a series of normal men, showed that when the urine flow was progressively increased from very low values the urea clearance also increased until the rate of urine excretion reached a value they termed the augmentation limit. At rates of urine excretion above the augmentation limit there was found to be no further increase in urea clearance. Subsequently this description has been accepted for dogs by Jolliffe and Smith[2] and Summerville, Hanzel and Goldblatt,[3] and it has been tacitly assumed by Van Slyke, Rhoads, Hiller and Alving[4] in their study of factors controlling urea excretion. A maximum urea clearance has also been posited by Dominguez[5] in his mathematical treatment of the relation existing between urea clearance and urine flow at rates of urine formation below the augmentation limit. The value fixed for the augmentation limit in the dog has been variously stated to be from 0.2 to 0.4 cc. per minute, depending in part upon the size of the animal, and also upon the level of the maximum clearance, which can be varied by changing protein intake.

Recent evidence has made it possible to accept the creatinine clearance as a measure of the rate of glomerular filtration in the dog. (Shannon,[6] Van Slyke, Hiller and Miller[7]), even at very high U/P ratios (Shannon[8]). The present observations were made to examine the relations between the rate of glomerular filtration and the rate of excretion of water, and the effect of these variables upon the urea clearance.

There is no standard of reference by which the clearances of

[1] Austin, J. H., Stillman, E., and Van Slyke, D. D., *J. Biol. Chem.*, 1921, **46**, 91.

[2] Jolliffe, N., and Smith, H. W., *Am. J. Physiol.*, 1931, **98**, 572; **99**, 101.

[3] Summerville, W. W., Hanzal, R. F., and Goldblatt, H., *Am. J. Physiol.*, 1932, **102**, 1.

[4] Van Slyke, D. D., Rhoads, C. P., Hiller, A., and Alving, A. S., *Am. J. Physiol.*, 1934, **109**, 336.

[5] Dominguez, R., *Am. J. Physiol.*, 1935, **112**, 529.

[6] Shannon, J. A., *Am. J. Physiol.*, 1935, **112**, 405.

[7] Van Slyke, D. D., Hiller, A., and Miller, B., *Am. J. Physiol.*, 1935, **113**, 611.

[8] Shannon, J. A., *Am. J. Physiol.*, 1936, **114**, 362.

various individual dogs can be converted to a common value. For this reason it was apparent beforehand that the data from each dog should be treated separately. We have taken cognizance of the fact that abstinence from water may change the physiological state of the animal, and consequently no experiment has been accepted that does not include a fair representation of urine flows over the entire physiological range. The experiments were conducted so that the rate of water excretion should return at some time during the observations as near to the initial state as possible; we believe that failure to observe these conditions is in part responsible for a misinterpretation of the true relation between rate of water excretion and urea clearance.

To date we have examined the urea and creatinine clearances in 405 periods in 5 dogs. Three of these dogs were observed upon a cracker meal, sucrose and lard maintenance diet, one upon a mixed diet, and one on both these diets at different times. Adequate vitamins were given to all animals and a salt mixture was used to supplement the low protein diet.

TABLE I.

Urea and creatinine clearances of Dog C., on mixed diet for 2 months prior to experiment. 1000 cc. of water were given at termination of period 2 and 500 cc. at termination of period 9. Creatinine was given 4 times in doses of 100, 50, 50 and 50 mg. per kilo. The presence of creatinine causes no change in the absolute urea clearance in relation to urine flow.

| Period No. | Duration min. | Urine Flow cc. per min. | —Plasma Level— Urea mg. % | Creatinine mg. % | ——Clearance—— Urea cc. per min. | Creatinine cc. per min. | Ratio Urea Clearance / Creat. Clearance |
|---|---|---|---|---|---|---|---|
| 1 | 36 | .191 | 39.4 | 9.52 | 29.2 | 95 | .307 |
| 2 | 34 | .20 | 38.5 | 9.26 | 27.8 | 98 | .285 |
| | | | 1 hr. 35 min. between periods | | | | |
| 3 | 31 | 7.23 | 30.6 | 8.52 | 70.3 | 123.5 | .570 |
| 4 | 31 | 5.42 | 28.6 | 8.15 | 65.5 | 117.6 | .557 |
| 5 | 31 | 1.71 | 26.7 | 7.62 | 54.0 | 107.8 | .501 |
| 6 | 37.5 | .613 | 25.4 | — | 41.6 | — | — |
| 7 | 34.5 | .275 | 24.2 | 9.17 | 38.6 | 111.0 | .348 |
| 8 | 41 | .268 | 24.0 | 9.52 | 36.8 | 110.6 | .333 |
| 9 | 42 | .191 | 23.9 | 8.52 | 26.8 | 101.2 | .265 |
| | | | 1 hr. between periods | | | | |
| 10 | 30 | 2.40 | 21.1 | 9.31 | 52.8 | 110 | .480 |
| 11 | 30 | .933 | 20.1 | 9.20 | 45.4 | 108.2 | .420 |
| 12 | 30 | .533 | 19.1 | 8.95 | 39.9 | 108 | .369 |

A typical experiment on one of the dogs is given in Table I. It is apparent that in this animal there is no point on the urea curve that can be designated as an "augmentation limit", in the sense of Austin, Stillman and Van Slyke.[1] There is a systematic increase in urea clearance with increasing urine flow throughout the entire range

of the latter. This increase in urea clearance is in part due to an increase in the amount of urea filtered (creatinine clearance) and in part due to diminished reabsorption of urea. In no 2 dogs that we have studied have either of these 2 factors been quantitatively the same. And in only one out of the 5 has the gross change in these 2 variables—rate of filtration and reabsorption of urea—been quantitatively so small as to produce an approximately constant urea clearance above 0.5 cc. per minute.

It may be noted that in any instance where the urea-creatinine clearance ratio changes with changing urine flow, the extraction ratio of both substances could not in theory be constant and independent of urine flow.

The theoretical treatment of urea clearances below the augmentation limit as proposed for the dog by Dominguez loses its significance in view of the fact that in many, and possibly most dogs there is no point that can be designated as the augmentation limit.

There is little or no change in the creatinine clearance in any dog in the ordinary experimental range of urine flows (between 0.5 cc. and 3 cc. per minute). At flows lower than 0.5 cc. per minute the clearance of this substance may or may not fall, depending apparently upon the degree of dehydration of the animal. The point at which this fall takes place is below 0.2 cc. per minute in 4 out of the 5 dogs studied, and is not reproducible in any one. We were unable consistently to obtain urine flows below 0.1 cc. per minute, due perhaps to the presence of large quantities of creatinine in the urine. In some experiments this rate of urine flow was reached with no fall in creatinine clearance. The irregular decrease in glomerular filtration may arise from the conditions of our experiments, since more dehydration is necessary to obtain low urine flows in the presence of creatinine than in its absence.

8416 P

# Stimulation of Oxygen Consumption and Suppression of Cell Division by Dihalo and Trihalophenols.

M. E. KRAHL AND G. H. A. CLOWES.

*From the Lilly Research Laboratories, Marine Biological Laboratory, Woods Hole, Mass.*

It is known that the oxygen consumption of fertilized sea urchin eggs *(Arbacia punctulata)* can be markedly stimulated by the addition of suitable concentrations of nitro and dinitrophenols.[1, 2] Stimulation of oxygen consumption in these cells can also be effected by certain oxidation-reduction indicators.[5, 6] In concentrations slightly greater than the optimum for respiration, certain nitrophenols block the cell division of fertilized Arbacia eggs. This division block is fully reversible since the eggs, when returned to sea water after a 3-hour exposure to concentrations many times the optimum for respiration, resume division and develop to swimming larvae.[1] In concentrations which are optimum for respiration, a number of oxidation-reduction indicators also inhibit cell division in fertilized Arbacia eggs, but the eggs so treated do not recover when they are returned to sea water.[5, 6]

Since the chemical and biological properties of the nitro compounds differ in almost every respect from those of the oxidation-reduction indicators,[5] it has been tentatively concluded that the nitro and dinitrophenols do not stimulate respiration or block cell division as a result of any possible oxidation or reduction which they may undergo in the cell. For the production of the respiratory-stimulating and division blocking effects, the unesterified phenolic OH group, accompanied by suitable substituents in the benzene ring, appears essential.[3]

The experiments presented here support this view since they show that, in fertilized eggs of Arbacia punctulata, oxygen consumption is stimulated and cell division is reversibly blocked by phenols containing no nitrogen and no substituent group capable of oxidation-reduction. These phenols contain, in addition to the OH group and the benzene ring, only chlorine, bromine, or iodine atoms. Phenols of this type are oxidized irreversibly at high positive potentials.[4]

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1 Clowes, G. H. A., and Krahl, M. E., *Science*, 1934, **80**, 384.

2 Clowes, G. H. A., Keltch, A. K., and Krahl, M. E., *Biol. Bull.*, 1935, **69**, 341.

3 Clowes, G. H. A., and Krahl, M. E., *J. Gen. Physiol.*, in press.

4 Fieser, L. F., *J. Am. Chem. Soc.*, 1931, **52**, 5204.

5 Krahl, M. E., and Clowes, G. H. A., *Biol. Bull.*, 1935, **69**, 340.

6 Runnström, J., *Biol. Bull.*, 1935, **68**, 327.

From preliminary experiments it appears that, in low concentrations, the monohalophenols do not stimulate oxygen consumption or reversibly block cell division; the 2,4-dihalophenols are very active in this respect; the 2,6-dihalophenols are inactive; the 2,4,5-trihalophenols are very active, while the 2,4,6-trihalophenols, in contrast to 2,4,6-trinitrophenol, stimulate oxygen consumption and block cell division at high dilution.[3]

Experiments were conducted according to methods described elsewhere.[3] Typical results are presented on a percentage basis in Table I. With each of the 3 reagents, the block to division was completely reversible, since all samples of eggs, after return to sea water, gave 90-100% development to top swimming larvae.

TABLE I.

Oxygen consumption and cell division of fertilized eggs of *Arbacia punctulata* in sea water containing various concentrations of 2,4-dichlorophenol (I), 2,4,5-trichlorophenol (II), and 2,4-dinitrophenol (III). Temperature 20° C. The values are expressed as percentages of the controls.

| Concentration of reagent moles per liter. | I | | II | | III | |
|---|---|---|---|---|---|---|
| | $O_2$ Uptake | Division | $O_2$ Uptake | Division | $O_2$ Uptake | Division |
| None—Control | 100 | 100 | 100 | 100 | 100 | 100 |
| $4x10^{-6}$ | 100 | 100 | 170 | 100 | 126 | 100 |
| $8x10^{-6}$ | 107 | 100 | 250 | 77 | 171 | 100 |
| $1.6x10^{-5}$ | 111 | 100 | 264 | 5 | 256 | 98 |
| $3.2x10^{-5}$ | 123 | 100 | 153 | 0 | 291 | 10 |
| $6.4x10^{-5}$ | 230 | 99 | 103 | 0 | 268 | 4 |
| $1.28x10^{-4}$ | 236 | 19 | 100 | 0 | 240 | 5 |
| $2.56x10^{-4}$ | 156 | 11 | 100 | 0 | 204 | 3 |
| $5.12x10^{-4}$ | 124 | 3 | 100 | 0 | 159 | 4 |

To ascertain the effects of dihalo and trihalophenols on mammals, preliminary experiments with a number of these compounds have been carried out in collaboration with Dr. K. K. Chen. After intravenous injection, 2,4-dichlorophenol did not increase the metabolic rate of rats or the body temperature of pigeons or dogs.

## 8417 C

### Effect of Anoxemia on Secretion of Urine in the Dog.

E. J. VAN LIERE, H. S. PARKER, G. R. CRISLER AND J. E. HALL.

*From the Department of Physiology, West Virginia University.*

Toth[1] has reported that the breathing of low oxygen tensions resulted in a marked diminution in the rate of urine secretion. In view of the fact that there is so little in the literature regarding the effect of anoxemia on urine secretion, it was thought worth while to report certain corroborative data which have been obtained in this laboratory from time to time in our work on anoxemia.

Twelve barbitalized dogs were used in our work, and a total of 50 experiments was made. Both ureters were cannulated, and the cannulae were attached to a glass Y tube, so arranged as to be independent of the skeletal movements of the animal. The drops of urine falling from the Y tube were counted by a drop recorder. In most instances simultaneous blood pressure tracings were made. Anoxemia was produced by allowing the dogs to breathe from an apparatus previously described[2] for mixing oxygen and nitrogen in the desired proportions.

In 45 of the 50 experiments the rate of urine secretion was markedly diminished. In 4 experiments the rate was unchanged, and in one experiment is was increased. In nearly every case the rate of secretion returned to above its normal level within 10 minutes after the anoxemia was discontinued. Table I gives a summary of all the results obtained.

TABLE I.

| % Oxygen | No. Trials | No. Diminished | No. Unchanged | No. Increased |
|---|---|---|---|---|
| 5.0 | 13 | 13 | 0 | 0 |
| 6.0 | 6 | 6 | 0 | 0 |
| 6.67 | 11 | 9 | 2 | 0 |
| 8.0 | 6 | 5 | 1 | 0 |
| 10.0 | 12 | 10 | 1 | 1 |
| 12.0 | 2 | 2 | 0 | 0 |
| Total | 50 | 45 | 4 | 1 |

The constant results obtained with the milder degrees of anoxemia indicate that the threshold for barbitalized dogs is above 12%

1 Toth, L. A., *Proc. Am. Physiol. Soc.*, 1935, **47**, 132.

2 Van Liere, E. J., *Am. J. Physiol.*, 1927, **82**, 727.

oxygen. The mechanism involved in this diminution in urine secretion is not fully understood, but in view of the work of Adolph[3] it is felt that the increased secretion of epinephrine may play a part. This, however, may not explain the fact that the depression in secretion was obtained almost immediately upon administering the anoxemia. It is possible that this sudden depression is due to vasoconstriction produced by a reflex nervous mechanism. The reflexes involving the kidney of the dog may well be different from those described by Adolph[3] in the frog, an amphibian. Of course, such a reflex nervous action could be followed and enhanced by the epinephrine action.

The supernormal phase which occurred in many instances may be due to a vasodilation, following the vasoconstriction in the kidneys. Such a reversal phenomenon is characteristic of epinephrine action.

It must also be pointed out that the hyperventilation produced by anoxemia causes changes in the reaction of the blood. It is possible that this is another factor which must be considered in the mechanism of the depression of urinary secretion by anoxemia.

*Summary.* Breathing of oxygen tensions of 5.0% to 12.0% caused a marked diminution in the urine secretion in 45 of 50 experiments upon 12 barbitalized dogs.

8418 P

## A Comparison of Effects of Stimulation of Right and Left Peripheral Vagus with Action of Acetylcholine on Electrocardiogram of the Cat.

OTIS M. COPE AND HELEN C. COOMBS.

*From the Department of Physiology and Biochemistry, New York Homeopathic Medical College and Flower Hospital.*

Electrocardiograms were taken in a series of experiments on 40 cats, in which nembutal was the anesthetic employed. The procedures were as follows:

1. Stimulation of the right and left peripheral vagus only; (20 cats.)
2. Jugular injection of acetylcholine (2 to 6 mg./kg.). (20 cats.)

[3] Adolph, E. F., *Am. J. Physiol.*, 1934, **108**, 177, and personal communication.

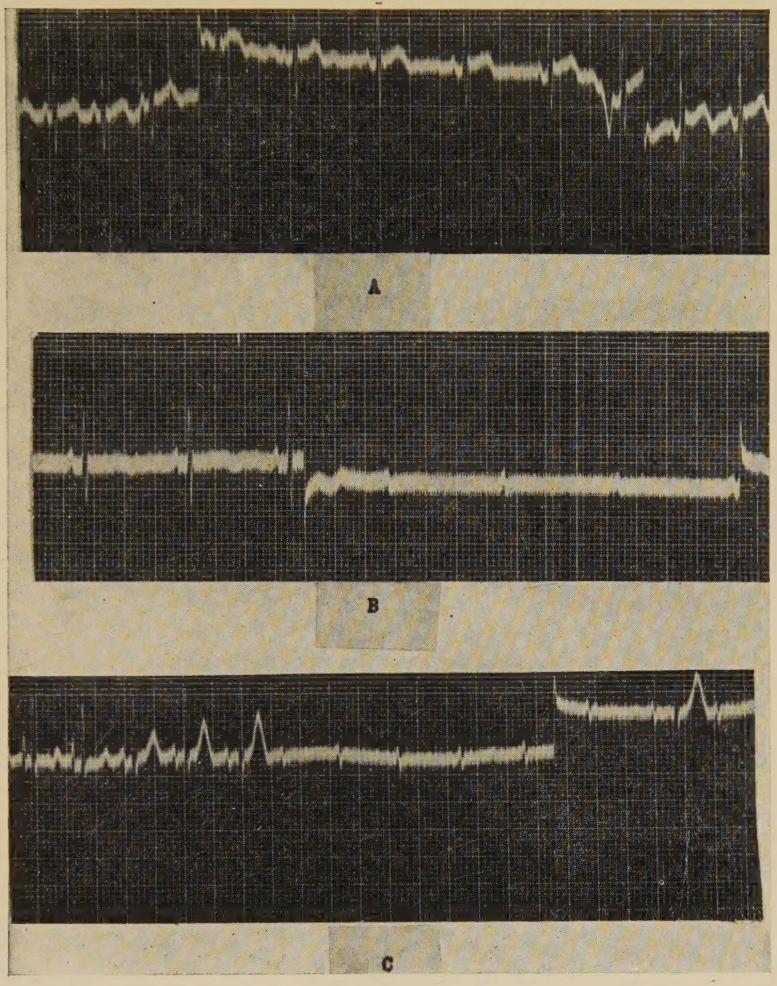


FIG. 1.

Electrocardiogram, lead II only. (a) Effect of stimulation of right vagus; (b) effect of stimulation of left vagus; (c) administration of acetylcholine. Note in (b) and (c) the persistence of P-waves and the dropping out of the QRS complex.

Typical electrocardiograms illustrate the following results:

1. Stimulation of the right vagus brings about a decrease in the rate of both P and QRS complexes, *i. e.*, a sinus bradycardia.
2. Stimulation of the left vagus brings about the dropping out of some QRS complexes, *i. e.*, a partial block.
3. Acetylcholine brings about (a) a decrease or temporary cessation, of QRS complexes; (b) a heavier dosage (sub-

lethal, about 6 mg./kg.) may result in temporary elimination of P-waves also.

Dale[1] has stated that "vagus impulses produce their effect by liberating acetylcholine among the fibers of the muscular wall of the heart." From these results it would seem that the action of acetylcholine on the heart more closely approximates that of the left, than of the right, vagus. The question is, therefore, raised as to whether some vagus fibers to the heart may not be more "cholinergic" than others, and a further differentiation of their activity on such a basis ultimately attempted.

[1] Dale, H. H., *Science*, 1934, **80**, 1.